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STATISTICAL CORRELATION OF PROTEIN AND RIBONUCLEIC ACID COMPOSITION

By George Gamow and Martynas Yčas

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Communicated September 29, 1955

Empirical evidence indicates with increasing clearness that ribonucleic acid (RNA) plays a vital role in protein synthesis. It appears rational to assume that the sequence of amino acids characterizing a given protein is uniquely determined by the sequence of nucleotides in the ribonucleic acid molecule.

While RNA is a polymer of four different nucleotides, proteins are polymers of 20 different amino acids. Since it is possible to form 20 kinds of triplets from four different elements, this suggests that each of the 20 amino acids is determined by a triplet of nucleotides, taken without regard to order.[1]

The fact that the internucleotide distances are comparable with the distances of amino acid residues in a protein when both are in the extended form makes it plausible that a given amino acid shares its determining nucleotides with neighboring amino acids. This would necessitate a correlation between neighboring residues in protein sequences, making certain pairs favored and others excluded (1, 2).

However, studies by Gamow, Rich, and Yčas (2) show that there does not appear to be any such interresidue correlation, and all sequences are apparently possible. Thus it appears more probable that the number of determining nucleotides exceeds by a factor of 3 the number of amino acid residues in the synthesized protein, so that neighboring residues do not share determining nucleotides.

It is not possible to test this hypothesis critically against available information on amino acid sequences, since all sequences are permitted. However, the model predicts that the statistical frequency of amino acid residues in proteins will show certain regularities. We have therefore attempted to test whether the distribution of amino acids predicted from the proposed model corresponds with analytically found distributions.

If one arranges the amino acids in a protein in order of abundance, repeats this on a collection of proteins,[2] and takes the mean values (without regard to identity) of the most abundant, second most abundant, third most abundant, etc., one obtains a curve shown in Figure 1. This will be referred to as a "distribution." In

order to see whether this curve corresponds to a random distribution, we compare it with a mathematical model obtained in the following way. A segment is divided into 20 sections at random, and the lengths of the longest, second longest, etc., are

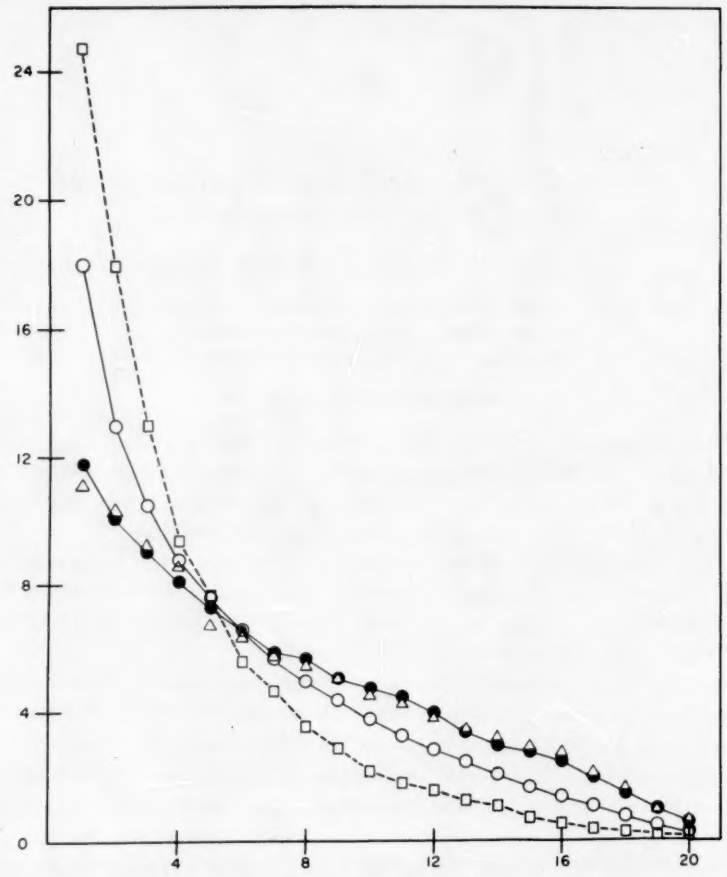

FIG. 1.—Distributions (as defined in text): ●, amino acids, 22 proteins; △, triplets from 7 nonviral RNA's; □, random triplets, Monte Carlo, 3,000 runs; ○, random distribution, $n = 20$, by von Neumann's formula; *abscissa*, ordinal rank; *ordinate*, relative frequency in per cent.

averaged over a large number of such divisions. This problem possesses an analytical solution for which we are indebted to John von Neumann. If the unit length is divided randomly into n sections and a_1, a_2, a_3, etc., are the mean lengths of the longest, second longest, etc., sections, then

$$a_j^{(n)} = \frac{1}{n}\left(\frac{1}{n} + \frac{1}{n-1} + \ldots + \frac{1}{n-j+1}\right).$$

The results given by this formula for $n = 20$ are likewise plotted in Figure 1. It is obvious that the distribution of amino acid residues in a collection of proteins deviates markedly from the random model distribution.

Assuming that the determination of amino acids is by nonoverlapping triplets, this deviation from randomness either originates in the translation procedure operating on a random distribution of nucleotides or is due to a deviation from randomness in the composition of the template itself.

In order to explore the first possibility, it was decided to go through the following Monte Carlo procedure: select four random fractions, normalized to one, and calculate the probabilities of the twenty different triplets. Assuming no bias, the relative frequency of each individual triplet will be given by the product of the frequencies of the component elements. It will be noted that there are three kinds of triplets of four different elements. Four contain three different elements (*abc*), sixteen contain two (*aab*), and four contain one (*aaa*). Since we consider triplets differing only in the order of elements as identical, the relative frequencies must further be multiplied by a weighting factor of 6 for the *abc* type, 3 for the *aab* type, and 1 for the *aaa* type. Repeat this procedure many times and average the amounts of the most abundant, second most abundant, and so on, triplets. This was done for us by Guilio Fermi and Nicholas Metropolis, using the electronic computer MANIAC of the Los Alamos Scientific Laboratory. The result of 3,000 runs is plotted in Figure 1. It will be noted that the curve deviates from the amino acid distribution curve even more than the previous theoretical one. Thus, if the triplet hypothesis is correct, the deviation from randomness of the amino acid distribution must arise from the nonrandom composition of the template.

The distribution, defined in the same way as for proteins, of 7 RNA's[2] is plotted in Figure 2, along with the random distribution expected from Neumann's formula with $n = 4$. The distribution of RNA, like that of protein, does indeed deviate markedly from the random. The attempt was made to see whether this deviation could be due to the fact, recently observed by Elson and Chargaff (3), that the total of adenine plus cytosine tends to equal the sum of guanine plus uracil. If this were the case, then the above-discussed random model should be modified in the following way. A unit length is divided into two halves and each half broken at random into two. According to the result obtained for us by S. Ulam, the means of the longest, second longest, etc., lengths must stand in the ratio $^5/_{12}$, $^4/_{12}$, $^2/_{12}$, and $^1/_{12}$. This is also plotted in Figure 2. Although this restriction brings the curve closer to the empirical one, the deviation is still marked. Thus the distribution of RNA does deviate essentially from random and in the same direction as the protein distribution.

Next, using the actual composition of the same 7 samples of RNA, the frequency of individual triplets was calculated for each RNA, the triplets arranged in decreasing order of magnitude, and the average values for the most abundant, second most abundant, etc., calculated. The results obtained (Fig. 1) coincide almost perfectly with the distribution of our sample of 22 proteins.

The same procedure was followed for 3 viruses, where the sample, although smaller, has the advantage of a presumably more direct relation of the RNA and protein. The results are similar to the previous ones, although the fit is less perfect (Figs. 3 and 4).

The above results seem to show that (1) the proportions of amino acids in proteins are not random; (2) this nonrandomness is not due to the application of the triplet translation procedure to a random RNA constitution; (3) the application of

the same translation procedure to the actual RNA composition leads to an excellent agreement with the observed amino acid distribution.

A further prediction from the model may be noted. Because of different weighting factors for triplets of the *abc*, *aab*, and *aaa* types, individual amino acids would be expected to be consistently abundant, rare, or of intermediate frequency within

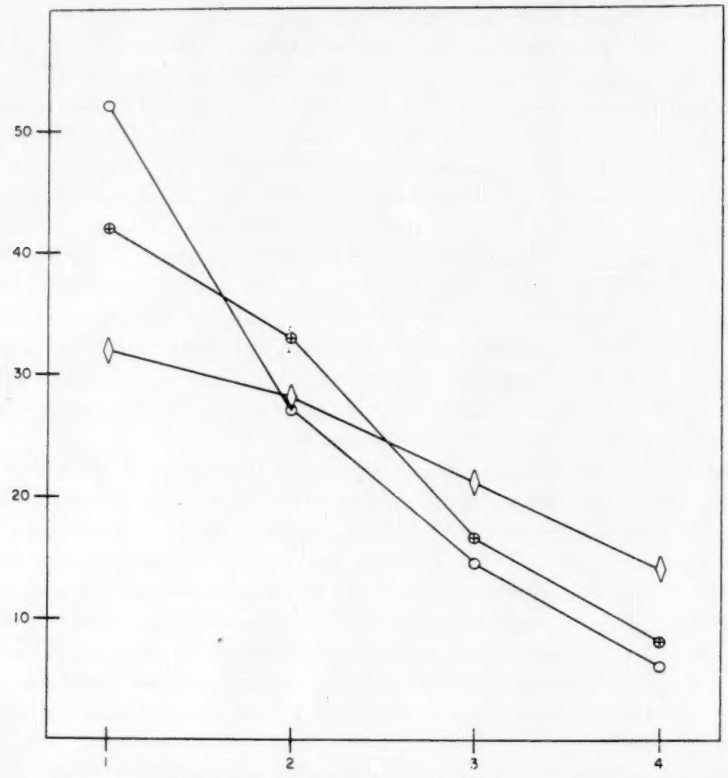

FIG. 2.—Distributions (as defined in text): ◇, 7 nonviral RNA's; ○, random distribution, $n = 4$, by von Neumann's formula; ⊕, random distribution if $A + B = C + D$ (after S. Ulan); *abscissa*, ordinal rank; *ordinate*, relative frequency in per cent.

wide limits of variation in composition of the RNA template. Tristram (4) has indeed observed this to be the case, each amino acid tending to be normally distributed about its characteristic frequency.

We consider that these results speak strongly in favor of the original hypothesis that amino acid residues in proteins are selected by independent triplets of nucleotides taken without regard to order.

It is our pleasant duty to express our thanks to G. Fermi, N. Metropolis, J. von Neumann, and S. Ulam for the help they have given us.

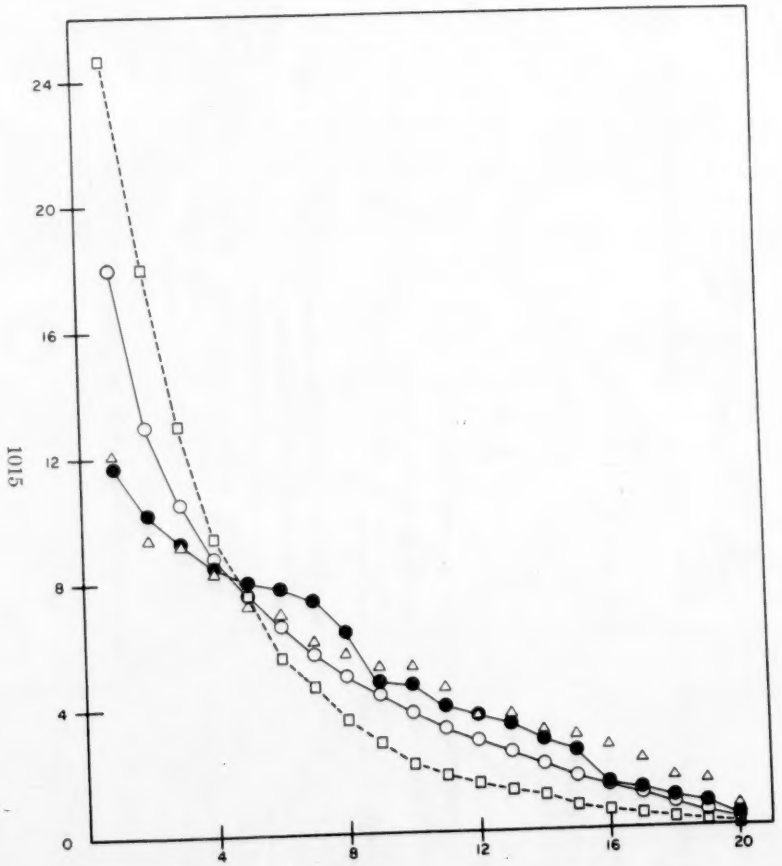

FIG. 3.—Same procedure as in Fig. 1, for 3 plant virus proteins.

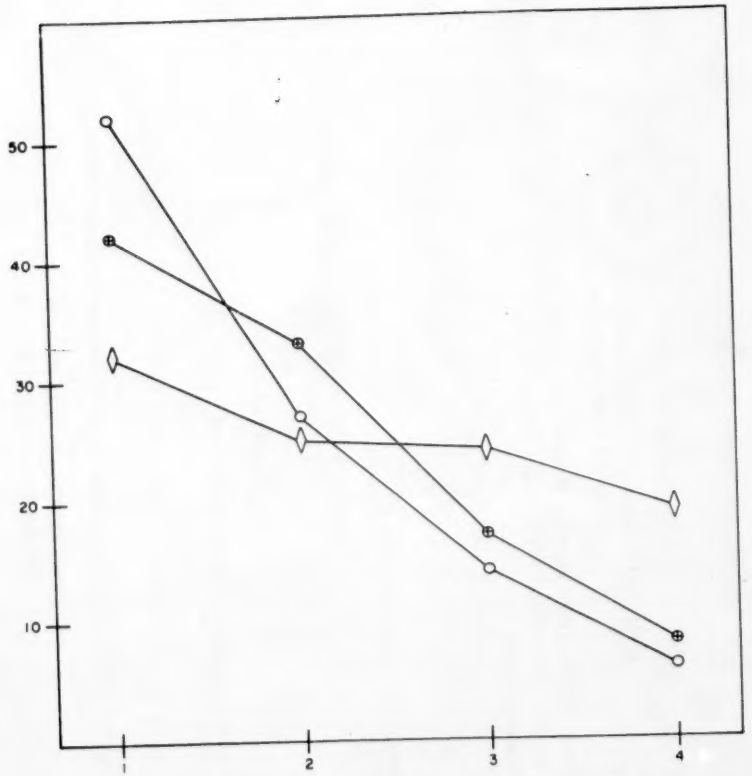

FIG. 4.—Same procedure as in Fig. 3, for 3 plant virus RNA's.

APPENDIX I

RANDOM DIVISION OF A UNIT LENGTH INTO n PARTS (AFTER J. VON NEUMANN)

Consider a unit length randomly divided into n parts so that the lengths of individual sections, as they follow from left to right, are $x_1, x_2, x_3, \ldots, x_n$. The values of x_i are subjected to the conditions

$$\sum_{i=1}^{n} x_i = 1 \qquad \text{and} \qquad 0 < x_i < 1.$$

Now let us define n numbers y_j as

$$\begin{aligned} y_1 &= \text{smallest of all } x\text{'s}, \\ y_2 &= 2^d \text{ smallest of all } x\text{'s}, \\ &\ldots\ldots\ldots\ldots \\ y_n &= \text{largest of all } x\text{'s}. \end{aligned}$$

The values of y_j are subject to the conditions

$$\sum_{j=1}^{n} y_j = 1 \qquad \text{and } 0 < y_1 < y_2 < \ldots < y_n < 1.$$

Considering the problem in n-dimensional space, we can use the statistical weight

$$\begin{aligned} d\pi &= dy_1\, dy_2 \ldots dy_{n-3}\, dy_{n-2}\, dy_{n-1} \\ &= dy_1\, dy_2 \ldots dy_{n-3}\, dy_{n-2}\, dy_n \\ &\ldots\ldots\ldots\ldots\ldots\ldots \\ &= dy_1\, dy_3\, dy_4 \ldots dy_{n-2}\, dy_{n-1}\, dy_n \\ &= dy_2\, dy_3\, dy_4 \ldots dy_{n-2}\, dy_{n-1}\, dy_n. \end{aligned}$$

The problem is to find the mean values of $y_1, y_2, \ldots, y_n$ for all possible divisions of the unit length. Put

$$z_j = y_j - y_{j-1} \qquad (j = 1, 2, \ldots, n).$$

Then, clearly,

$$y_j = \sum_{k=1}^{k=j} z_k,$$

and the restricting conditions on z_k become

$$nz_1 + (n-1)z_2 + \ldots + z_n = 1; \qquad z_k > 0.$$

The weight $d\lambda$ will now be proportional to

$$\begin{aligned} d\lambda &= dz_1\, dz_2 \ldots dz_{n-3}\, dz_{n-2}\, dz_{n-1} \\ &= dz_1\, dz_2 \ldots dz_{n-3}\, dz_{n-2}\, dz_n \\ &\ldots\ldots\ldots\ldots\ldots\ldots \\ &= dz_1\, dz_3\, dz_4 \ldots dz_{n-1}\, dz_n \\ &= dz_2\, dz_3\, dz_4 \ldots dz_{n-1}\, dz_n. \end{aligned}$$

Put

$$\omega_k = (n + 1 - k)z_k \; (k = 1, 2, \ldots, n).$$

Then the restrictions become

$$\sum_{k=1}^{n} \omega_k = 1, \qquad \omega_k > 0,$$

and the statistical weight is proportional to

$$\begin{aligned} d\mu &= d\omega_1\, d\omega_2 \ldots d\omega_{n-3}\, d\omega_{n-2}\, d\omega_{n-1} \\ &= d\omega_1\, d\omega_2 \ldots d\omega_{n-3}\, d\omega_{n-2}\, d\mu_n \\ &\quad \ldots\ldots\ldots\ldots\ldots \\ &= d\omega_1\, d\omega_3\, d\omega_4 \ldots d\omega_{n-1}\, d\omega_n \\ &= d\omega_2\, d\omega_3\, d\omega_4 \ldots d\omega_{n-1}\, d\omega_n. \end{aligned}$$

because of the symmetry of restricting conditions in ω-space,

$$\bar{\omega}_1 = \bar{\omega}_2 = \ldots = \bar{\omega}_n,$$

and, since

$$\sum_{k=1}^{k=n} \omega_k = 1,$$

then

$$\bar{\omega}_1 = \bar{\omega}_2 = \ldots = \bar{\omega}_n = \frac{1}{n}.$$

Therefore,

$$\bar{z}_k = \frac{\bar{\omega}_k}{n+1-k} = \frac{1}{n} \cdot \frac{1}{n+1-k}$$

and

$$\bar{y}_j = \sum_{k=1}^{k=j} \bar{z}_k = \sum_{k=1}^{k=j} \frac{1}{n} \cdot \frac{1}{n+1-k},$$

$$\bar{y}_j = \frac{1}{n}\left(\frac{1}{n} + \frac{1}{n-1} + \ldots + \frac{1}{n-j+1}\right),$$

as given in the text.

APPENDIX II

RANDOM DIVISION OF A UNIT LENGTH INTO FOUR PARTS UNDER THE CONDITION THAT THE FIRST DIVISION IS MADE IN THE MIDDLE

(AFTER S. ULAM)

Consider a unit length broken into two halves, I and II, and each of these again broken randomly into two. Let x be the longest part of I and y the longest part of II. Then the distributions of both x and y are uniform in the interval $1/4$ to $1/2$. We plot x and y in a two-dimensional diagram (Fig. 5) and pick at random a point P within this square. If the point is above the diagonal (as shown in the diagram), we take its y co-ordinate (because it is larger than x), and if it is below the diagonal, we take its x co-ordinate (which is, in this case, largest). The center of gravity of the triangle ULA has the co-ordinate $y = 1/4 + 2/3 \cdot 1/4 = 5/12$, and the same figure gives the x co-ordinate of the triangle AMU. This gives us the mean length of the longest piece.

The second longest piece will be the shorter of the two x or y. This will correspond to the x co-ordinate of the center of gravity of ULA, or the y co-ordinate of the center of gravity of AMU, and is equal to $^1/_4 + {}^1/_3 \cdot {}^1/_4 = {}^4/_{12}$.

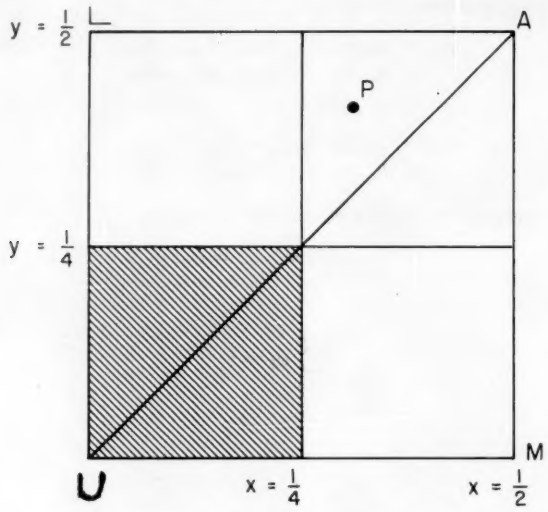

FIG. 5—Graphical solution of restricted division problem.

The shortest of all pieces will correspond to a point in the shaded area of Figure 5. If the point in this area is above the diagonal, we take its x co-ordinate, and if it is below, its y co-ordinate. In each case it is equal to $^1/_3 \cdot {}^1/_4 = {}^1/_{12}$, which gives the mean length of the shortest piece. The length of the next shortest is, of course, $1 - {}^5/_{12} - {}^1/_{12} = {}^2/_{12}$. Thus the four mean lengths will be

$$^5/_{12};\ ^4/_{12};\ ^2/_{12};\ ^1/_{12}$$

as given in the text.

[1] One of us has previously suggested (1) that the coincidence of the number 20, which is the number of combinations of four different things taken three at a time and the number, also 20, of the different kinds of amino acids occurring in proteins is not accidental. Our belief that this is the case is strengthened by certain biochemical findings. Besides the 20 commonly occurring amino acids, certain others are found as components of one or a few proteins (2). In three cases something is known of their mode of formation. The present evidence strongly indicates that thyroxin (5), hydroxyproline (6), and phosphoserine (7) are formed from tyrosine, proline, and serine residues already incorporated into protein. This is in contrast with the 20 commonly occurring amino acids, which are incorporated as such. Thus the evidence to date does not make it necessary to assume that template configurations for selecting more than 20 amino acids exist.

[2] We have used data on the amino acid composition of 22 proteins: whale myoglobin, horse hemoglobin, aldolase, triosephosphate dehydrogenase, phosphorylase, zein, ovalbumin, β-lactoglobulin, α-casein, conalbumin, fibrinogen, bovine serum albumin (4); prothrombin (8); carboxypeptidase (9); papain (10); ribonuclease (11); ACTH (12); insulin (13); tropomyosin, actin (14); barley β-globulin (15); and lysozyme (16). Virus proteins: tobacco mosaic (17), turnip yellow (18), and tomato bushy stunt (19). Since analysis of a chemical hydrolyzate of a protein does not distinguish glutamine from glutamic acid, or asparagine from aspartic acid, we have estimated the abundance of these amino acids as follows: the published amide content of each protein was

assigned to glutamine and asparagine in the ratio in which glutamic and aspartic acid occur in the chemical hydrolyzate. This procedure was unnecessary for insulin, ACTH, lysozyme (20), and zein, where the actual content of the four acids is known. The data on the amide content of tobacco mosaic virus protein are from Schramm (21). No data are available on the amide content of turnip yellow virus protein, and we estimate the content to be the mean of that of tobacco mosaic and tomato bushy stunt viruses. This value is obviously very uncertain. The RNA composition data are taken from the compilation of Magasanic and refer to the composition of the RNA of calf liver, calf spleen, carp nucleotropomyosin, cat brain, sea urchin eggs, yeast, and *Escherichia coli* (22). Virus RNA data: tobacco mosaic (23), turnip yellow (24), and tomato bushy stunt (19). The nucleoide and amino acid compositions of RNA and protein are expressed in moles per cent.

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*CRYSTALLIZATION OF PURIFIED MEF-1 POLIOMYELITIS VIRUS PARTICLES**

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Communicated by W. M. Stanley, October 20, 1955 and read before the Academy, November 3, 1955

Highly purified preparations of poliomyelitis virus, strain MEF-1, have been shown by analytical ultracentrifugation to contain only one homogeneous component.[1] Electron microscopic and other studies have revealed this component to consist of spherical particles approximately 27 mμ in diameter which are readily aligned in two-dimensional, close-packed arrays and with which infectivity is associated.[1, 2] The present report is concerned with the preparation and the description of properties of three-dimensional crystals, readily visible in the light microscope, which were obtained from a highly purified and concentrated preparation of such virus particles. Although several plant viruses have been crystallized[3] since the initial crystallization of tobacco mosaic virus by Stanley in 1935,[4] this is believed to be the first report of the crystallization of a virus affecting man or animals.

Crystallization.—The source of virus was the fluid harvested from monkey kidney tissue cultures infected with strain MEF-1 poliomyelitis virus and provided by the Connaught Laboratories, Toronto. The initial purification procedure, which has been previously outlined,[1] included methanol precipitation, butanol extraction, ultracentrifugation, nuclease digestion, and electrophoretic fractionation. Further purification of the preparation was achieved by sedimentation through a sucrose density gradient in a swinging cup rotor (Spinco SW-39), as described by Brakke for plant viruses.[5] In this way approximately 1.2 mg. of nucleoprotein material was obtained as a single sedimenting band in 0.73 ml. of 0.14 *M* NaCl containing approximately 30 per cent sucrose. This represented the virus concentrated from 15 liters of infected monkey kidney tissue culture fluid. The preparation was diluted to 2.0 ml. with 0.14 *M* NaCl and was sedimented by ultracentrifugation in an average centrifugal field 100,000 $\times$ *g* for 2.5 hours. The clear, gel-like pellet was covered with 0.3 ml. of unbuffered 0.14 *M* NaCl at pH 5.9 and gently rocked overnight at 4° C. After this treatment the pellet appeared white and opaque, and upon suspension discrete crystals were seen.

After removing a small fraction of the suspended crystals for further observation, the mother-liquor was removed from the remaining crystals. The latter were washed twice with isotonic saline and the washings pooled with the mother-liquor. The crystals were readily dissolved by warming to room temperature in 0.14 *M* NaCl adjusted to pH 8.5 with dilute NaOH.

The virus was recrystallized as follows: An aliquot of the dissolved crystals was diluted with a small volume of 0.14 *M* NaCl buffered with 0.001 *M* phosphate at pH 7.6, and the virus was then sedimented in a sealed capsule[6] in the swinging cup rotor of the ultracentrifuge. After removal of the supernatant fluid, the pellet was suspended in a small volume of isotonic saline buffered with 0.01 *M* phosphate at pH 5.9 and allowed to stand at 4° C. Small crystals were seen by microscopic examination. After low-speed centrifugation, the mother-liquor was removed from the re-

crystallized material, which was washed with pH 5.9 saline The washings were pooled with the mother-liquor. These crystals again were easily dissolved in isotonic saline buffered at pH 7.8 with 0.01 *M* phosphate.

Properties of the Crystals.—A series of photomicrographs of the crystals taken in ultraviolet light at various wave lengths is shown in Figure 1. The rapid decrease in transmission between 295 and 280 mμ is consistent with the nucleoprotein nature of the virus particles and their ultraviolet absorption spectrum in suspension.[1] The photomicrograph in visible light shown in Figure 2 gives some idea of the shape of the crystals. They appear to be tetragonal prisms with pyramids at both ends. The larger specimens measure approximately 30 μ in length.

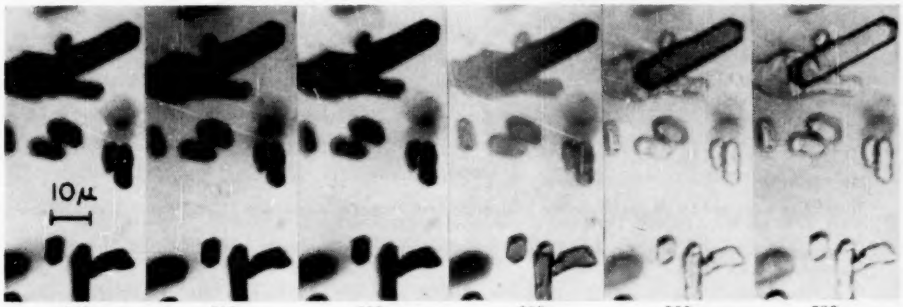

FIG. 1.—Crystals of MEF-1 poliomyelitis virus particles photomicrographed at various wave lengths in the ultraviolet.

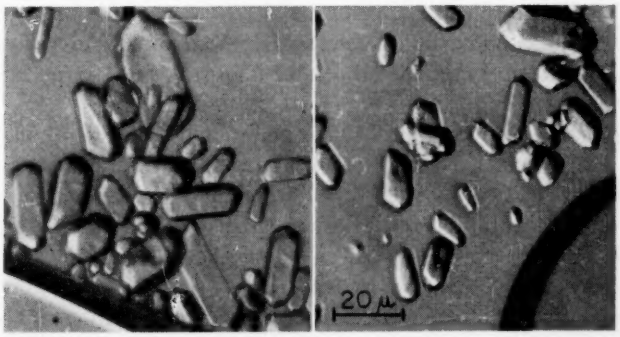

FIG. 2.—Crystals of MEF-1 poliomyelitis virus particles photomicrographed in visible light.

Evidence that the crystals contained the virus particles was provided by the essentially constant specific infectivity measurements of solutions of washed crystals, of mother-liquors, and of the two fractions of purified and concentrated virus from which the crystals were derived. Infectivity measurements were made by plaque assay on monkey kidney cultures by a modification[7] of the technique of Dulbecco and Vogt.[8] Specific infectivities were expressed as the number of plaque-forming units (PFU) per unit mass of nucleoprotein. The latter was measured by optical density at 260 mμ in a Beckman spectrophotometer and in some instances by direct

chemical analysis for nitrogen. By means of analytical electron microscopy, an estimate was also made of the ratio of countable physical particles to PFU.[9] Since there was no electron microscopic evidence of material other than the 27-mμ particles in the dissolved crystals, it was possible to estimate the mass of a single physical particle from the experimental data. The results of various experiments are summarized in Table 1.

TABLE 1

SPECIFIC INFECTIVITIES* OF VARIOUS PURIFIED AND CRYSTALLINE FRACTIONS OF MEF-1 VIRUS

Fractions	Relative Sequence of Fractionation	PFU/Ml per Unit OD†	PFU per Gram‡	27-mμ Particles per PFU	Estimated Grams‡ per Particle
Electrophoretic	1	2.2×10^9	1.6×10^{13}	3,000	2.1×10^{-17}
Density gradient	2	3.1×10^9	....	....	....
Crystalline virus	3	3.3×10^9	2.2×10^{13}	2,250	2.0×10^{-17}
Mother-liquor from 1st crystallization	4	3.2×10^9	2.3×10^{13}	1,300	3.3×10^{-17}
Recrystallized virus	5	3.5×10^9	....	850	....
Mother-liquor from recrystallization	6	3.4×10^9	....	1,200	....

* Infectivity measured by plaque assay (see Fogh and Lund, *Proc. Soc. Exptl. Biol. Med.* [in press], and Dulbecco and Vogt, *J. Exptl. Med.*, **99**, 167, 1954) and expressed as plaque-forming units (PFU).

† $OD_{260m\mu}^{1\ cm.}$ = optical density measured at 260-mμ wave length for a solution 1 cm. thick.

‡ Nitrogen × 6.25.

Discussion.—The constant specific infectivity values listed in the third and fourth columns of Table 1 for the density gradient, crystalline, and mother-liquor fractions attest to the comparable degrees of purity of these fractions. In the fifth column, the reciprocal specific infectivities expressed as physical particles per PFU are more variable. This probably can be accounted for by a variable loss of physical particles by adsorption and disintegration experienced in the preparation, dilution, and spraying of extremely small samples of highly purified virus for quantitative electron microscopy.

Physical and chemical data obtained earlier on highly purified though not crystalline MEF-1 virus provided sufficient information to estimate by Stokes's low a virus-particle mass of $1\text{–}1.5 \times 10^{-17}$ gm.[1] The mass of the particles is also calculable from the nucleoprotein and physical-particle concentrations of the purified virus preparations determined by direct chemical analysis and analytical electron microscopy, respectively. If the physical-particle count is low, owing to losses discussed above, the value of the mass per particle (sixth column) will be high. The difference between the particle mass estimated from Stokes's law and the 2.0×10^{-17} gm. obtained for the particles from crystalline virus can probably be attributed, therefore, to low electron microscope counts. The above data, supported by ultracentrifugal and electron microscopic evidence for homogeneity, lead to the conclusion that the crystals were composed of the 27-mμ particles.

It is probable that a large percentage of the particles in the crystalline state are inactive due to prolonged storage of the virus. That fraction which is inactive is difficult to determine because of our uncertain knowledge of the efficiency of the plaque-assay technique.[7] It is possible, however, that essentially all the physical particles were at one time mature, viable virus and that biological inactivation resulted in no physical and chemical alteration detectable by presently available tests.

This idea is compatible with the ability of the particles to crystallize, since it has been generally observed that no denatured protein will crystallize.[10]

Preliminary experiments in our laboratory with purified Saukett poliomyelitis virus indicate that it can also be crystallized under conditions successfully applied to the MEF-1 virus. It does not seem unreasonable, therefore, to expect all three types of human poliomyelitis as well as other animal viruses of small size to be capable of crystallization under proper conditions. This crystallization of a virus affecting man and animal emphasizes anew certain basic similarities between animal and plant viruses.

Summary.—The crystallization of human poliomyelitis virus particles from a highly purified and concentrated preparation has been reported for the first time. The appearance of the crystals and the evidence that the crystals are composed of characteristic virus particles, 27 mμ in diameter, are presented and discussed.

The authors are indebted to Drs. Russell L. Steere and Robert C. Backus for the visible and ultraviolet photomicrographs, respectively, of the virus crystals, and to Miss Joan Sprecher and Miss Helen Fisher for their excellent technical assistance.

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*INFLUENCE OF AUXIN ON CELL-WALL METABOLISM**

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INTRODUCTION

The plant hormone auxin (indole-3-acetic acid, IAA) controls rate of increase in size of the cells of many plant tissues. Thus, excised sections of the oat coleoptile respond to addition of the hormone by increasing more rapidly in length. It is known that the auxin-induced increase in cell volume of the oat section is immediately due to the uptake of water by the cell.[1] Auxin-induced uptake of water by the coleoptile is in turn an osmotic phenomenon.[2] Net water uptake by the coleop-

tile section takes place when the diffusion-pressure deficit of the section, DPD_i, is greater than the diffusion-pressure deficit of the external solution with which the section is in contact, DPD_o. DPD_i is in turn determined by the relation

$$DPD_i = \text{Osmotic concentration of cell contents} - \text{Wall pressure.} \quad (1)$$

It has been indicated[2] that in the oat coleoptile section, auxin may increase DPD_i by decreasing the wall-pressure term of equation (1). This conclusion, arrived at by study of the osmotic relations of the coleoptile, was reached by Heyn in 1931 on the basis of measurements of tissue plasticity as influenced by auxin.[3] The problem of how auxin increases tissue growth rate is then the problem of how auxin brings about relaxation of cell-wall pressure. In the present communication it will be shown that auxin influences a particular facet of the metabolism of the cell wall of the coleoptile. It has been found that the methyl carbon of C^{14}-methyl-labeled methionine is incorporated into cell-wall material. The rate of methyl carbon incorporation into a particular portion of the cell-wall material, the hot-water-soluble portion, is increased in the presence of auxin. Much of the carbon incorporated is removable in a manner characteristic of methyl ester groups. There is also an auxin-induced increase in the rate of incorporation of glucose-derived carbon into the hot-water-soluble fraction. It is suggested that the physical properties of the cell wall may be affected by the hormonally influenced methyl carbon incorporation into and synthesis of particular cell-wall components. The experimental finding is of interest, since it constitutes a metabolic effect of the auxin which is detectable within short time periods.

MATERIALS AND METHODS

The material for the experiments described below consisted of 5-mm. sections cut 3 mm. below the tip of coleoptiles of oat (variety Siegeshafer) seedlings grown in the conventional manner.[4] Primary leaves were removed from all sections. Incorporation of the carbon of varied substances into the cell wall of the section tissue was followed by incubation of sections in the desired C^{14}-labeled substrates, which were obtained from the California Foundation for Biochemical Research. Two hundred sections floating on 4.5 ml. of solution were used for each treatment of each experiment. Solutions were buffered with 0.0025 *M* potassium maleate, pH 4.8; IAA, 5 mg. per liter, was used as the auxin where indicated. At the end of the desired incubation period, the sections were rinsed with water and ground rapidly in a mortar, and the water-soluble and protoplasmic materials were separated from the cell-wall debris by repeated washing and filtration with cold water on a sintered glass funnel. The washed cell-wall material was then separated into constituent groups of components by the procedure outlined in Figure 1, which is adapted from Sinclair and Crandall[5] and Bonner.[6] The names classically applied to the cell-wall fractions of different solubilities are included in Figure 1, but we have no rigorous evidence that the fractions prepared from our material are, in fact, the classical cell-wall materials.

Aliquots of the hot-water-soluble, hot-dilute-acid-soluble, and hot-ammonium-oxalate-soluble extracts of the wall material were counted on copper planchets.

The alkali-soluble fractions were neutralized with 0.5 *N* and 4 *N* or 9 *N* sulfuric acid before plating. The radioactivities of fractions described in Tables 3 and 5

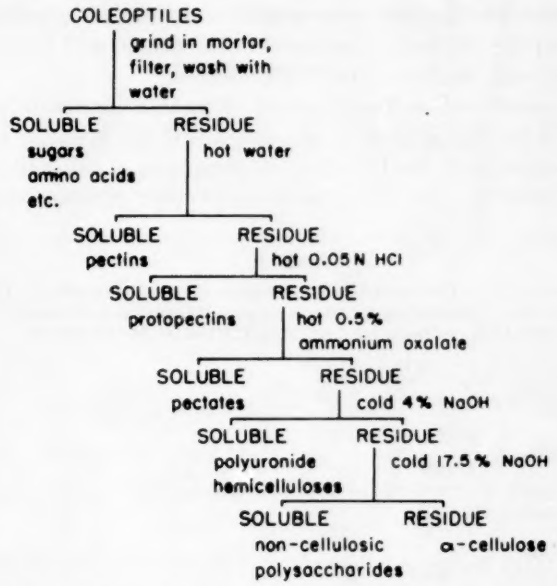

FIG. 1.—Fractionation procedure used for the separation of cell-wall components of oat coleoptile sections.

were counted with a thin-window Geiger-Müller tube. The other experiments were counted in an atmosphere of *Q* gas with a micromil window tube. All counts are corrected to infinite thinness.

EXPERIMENTAL RESULTS

It has been shown by Sato[7] that the methyl group of methionine provides the methyl ester group of the pectic substances of roots. That the carbon of C^{14}-methyl-labeled methionine is rapidly incorporated into the cell-wall constituents of the oat coleoptile is shown by the data of Table 1. When coleoptile sections are incubated in methyl-labeled methionine, the hot-water-soluble, dilute-acid-soluble, ammonium-oxalate-soluble, and dilute-alkali-soluble fractions of the wall exhibit extensive labeling within 3–4 hours. Other data show that the rate of labeling is approximately constant with time over this period. The cell-wall fractions which are extensively labeled constitute the pectin, protopectin, pectate, and polyuronide fractions of classical cell-wall chemistry.[6] Relatively little labeling is found in the portion soluble only in concentrated alkali or in the alkali-insoluble residue, which includes the noncellulosic polysaccharide and cellulose of the wall. That a portion of the C^{14} incorporated into the wall of the oat coleoptile may be located in methyl ester groups is indicated by the fact that the specific activity of the hot water and dilute-acid-soluble fractions is reduced by 90 per cent or more by hydroly-

sis for 60 minutes in 0.1 N NaOH at room temperature, a procedure which de-esterifies the carboxyl group of pectic material.

The data of Table 1 show in addition that the incorporation of the C^{14} of methyl-labeled methionine into the hot-water-soluble and ammonium-oxalate-soluble fractions of the coleoptile cell wall is increased in the presence of IAA. Incorporation into the other cell-wall fractions is but little affected.

That the increased rate of incorporation of methyl carbon into the hot-water-soluble portion of the wall is actually associated with the presence of IAA and is not an artifact associated with the IAA-induced expansion of cell-wall area is indicated by the data of Table 2. For this type of experiment, sections were supplied with

TABLE 1

INCORPORATION OF C^{14} FROM $C^{14}H_3$-METHIONINE INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS IN THE PRESENCE AND ABSENCE OF IAA (4-HOUR INCUBATION, 0.00133 M METHIONINE)*

CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA	CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA
Hot water soluble	732	1,010			
Hot 0.05 N HCl soluble	3,490	3,660	17.5 per cent NaOH soluble	81	83
Hot 0.5 per cent ammonium oxalate soluble	118	335	Residue	21	23
4 per cent NaOH soluble	364	261	Total	4,806	5,372

* Initial specific activity of culture solution: −IAA, 74.6 × 10^6 cpm/mM; +IAA, 81.2 × 10^6 cpm/mM.

TABLE 2

INCORPORATION OF C^{14} FROM $C^{14}H_3$-METHIONINE INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS IN THE PRESENCE AND ABSENCE OF IAA (3-HOUR INCUBATION, 0.3 M MANNITOL, 0.00025 M METHIONINE)*

CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA	CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL −IAA	+IAA
Hot water soluble	300	551			
Hot 0.05 N HCl soluble	2,350	2,175	17.5 per cent NaOH soluble	31	12
Hot 0.5 per cent ammonium oxalate soluble	57	56	Residue	12	19
4 per cent NaOH soluble	366	342	Total	3,116	3,155

* Initial specific activity of culture solution: −IAA, 279 × 10^6 cpm/mM; +IAA, 262 × 10^6 cpm/mM.

C^{14}-methyl-labeled methionine in the presence or absence of IAA but in the presence of a nonabsorbable solute, mannitol, supplied in such a concentration (0.3 M) as to just suppress growth of the sections. The data of Table 2 show that in the presence of IAA the rate of incorporation of methyl carbon into the hot-water-soluble fraction of the wall is substantially increased. Incorporation into other fractions of the wall is not influenced by IAA under the conditions of the experiment of Table 2.

It appears that the methyl-derived carbon of the hot-water-soluble and dilute-alkali-soluble fractions of the coleoptile wall is subject to metabolic turnover. This

is indicated by the results of the experiment of Table 3. In this type of experiment, sections were first incubated for 4 hours in methyl-labeled methionine. At the end of this time they were rinsed for 1 hour in water and were then incubated for an additional 17 hours in a high concentration of unlabeled methionine. The exist-

TABLE 3

INCORPORATION OF C^{14} FROM $C^{14}H_3$-METHIONINE INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS (4-HOUR PRETREATMENT, 0.00133 *M* $C^{14}H_3$-METHIONINE, −IAA; 17-HOUR INCUBATION, 0.04 *M* NONRADIOACTIVE METHIONINE, ±IAA)*

CELL-WALL FRACTION	CPM/10^5 CPM SUBSTRATE ABSORBED/200 SECTIONS: After 4-Hr. Pretreatment	After 17-Hr. Incubation: −IAA	After 17-Hr. Incubation: +IAA
Hot water soluble	952	540	740
Hot 0.05 *N* HCl soluble	6,530	5,950	5,950
Hot 0.5 per cent ammonium oxalate soluble	383	291	453
4 per cent NaOH soluble	1,010	567	1,118
17.5 per cent NaOH soluble	406	187	288
Residue	26	12	22
Total	9,307	7,547	8,571

* Initial specific activities of culture solutions: pretreatment, 64.8 × 10^6 cpm/mM; −IAA, 67.9 × 10^6 cpm/mM; +IAA, 63.5 × 10^6 cpm/mM.

ence of metabolic turnover should be indicated by a decrease in the amount of C^{14} in the wall or in its fractions during the second incubation period. The data of Table 3 show that in the absence of IAA about one-half of the methyl-carbon-derived activity of the hot-water-soluble and dilute-alkali-soluble portions of the wall is lost during the second incubation period. This decorporation of methyl-derived carbon is depressed in the presence of IAA.

The effect of IAA in increasing the rate of methyl carbon incorporation into the hot-water-soluble portion of the cell wall is paralleled at least in part by the effect

TABLE 4

INCORPORATION OF C^{14} FROM GLUCOSE-C^{14} INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS IN THE PRESENCE AND ABSENCE OF IAA (3-HOUR INCUBATION, 0.3 *M* MANNITOL, 0.00004 *M* GLUCOSE)*

CELL-WALL FRACTION	CPM/10^6 CPM SUBSTRATE RETAINED/MG. DRY CELL WALL: −IAA	+IAA
Hot water soluble	977	1,093
Hot 0.05 *N* HCl soluble	9,660	9,300
Hot 0.5 per cent ammonium oxalate soluble	69	74
4 per cent NaOH soluble	1,613	1,395
17.5 per cent NaOH soluble	568	375
Residue	428	428
Total	13,315	12,665

* Initial specific activity of culture solution: −IAA, 2,770 × 10^6 cpm/mM; +IAA, 2,470 × 10^6 cpm/mM.

of IAA on the incorporation of the carbon of C^{14}-labeled glucose into the same fraction. The data of Table 4 concern an experiment in which sections were incubated for 3 hours in glucose uniformly labeled with C^{14}, in the presence of 0.3 *M* mannitol. This type of experiment is comparable to that of Table 2, in that the effect of IAA

on incorporation into the cell wall is measured in the absence of complications which might be introduced by effects of growth itself on cell-wall synthesis. The data of Table 4 show that the carbon of C^{14}-labeled glucose is rapidly and extensively incorporated into all the cell-wall fractions. The presence of IAA appears to increase the rate of incorporation into the hot-water-soluble portion. Incorporation into the other fractions of the wall is either depressed or unaffected by the presence of the hormone.

The data of Table 5 concern an experiment in which sections were incubated for 17 hours in an excess of unlabeled glucose after a 3-hour pretreatment in C^{14}-labeled glucose. These data indicate that the glucose-derived carbon of the cell wall, particularly that of the hot-water-soluble and concentrated-alkali-soluble portions,

TABLE 5

INCORPORATION OF C^{14} FROM GLUCOSE-C^{14} INTO CELL-WALL FRACTIONS OF OAT COLEOPTILE SECTIONS (3-HOUR PRETREATMENT, 0.000167 *M* GLUCOSE-C^{14}, −IAA; 17-HOUR INCUBATION, 0.09 *M* NONRADIOACTIVE GLUCOSE ±IAA)*

CELL-WALL FRACTION	CPM/10^5 CPM SUBSTRATE ABSORBED/200 SECTIONS		
	After 3-Hr. Pretreatment	After 17-Hr. Incubation −IAA	After 17-Hr. Incubation +IAA
Hot water soluble	1,077	562	956
Hot 0.05 *N* HCl soluble	14,268	13,901	14,087
Hot 0.5 per cent ammonium oxalate soluble	380	460	352
4 per cent NaOH soluble	4,137	4,754	3,721
17.5 per cent NaOH soluble	1,327	823	1,951
Residue	1,492	1,704	1,751
Total	22,681	22,204	22,818

* Initial specific activities of all culture solutions: 580×10^6 cpm/mM.

may be subject to metabolic turnover. This turnover is decreased in the presence of IAA. The bulk of the glucose-derived carbon of the wall does not, however, appear to be subject to turnover. This indicates that the incorporation of glucose carbon represents, in general, net synthesis of cell-wall material, most of which occurs independently of auxin.

SUMMARY

1. The carbon atoms of C^{14}-methyl-labeled methionine and of glucose uniformly labeled with C^{14} are rapidly incorporated into cell-wall constituents of oat coleoptile sections.

2. Incorporation of the carbon of methyl-labeled methionine or of glucose into a particular fraction of the wall, the hot-water-soluble portion, is enhanced in the presence of the plant hormone indole-3-acetic acid (IAA) even if the IAA-induced growth of the tissue is suppressed by a suitable external osmotic concentration.

3. Methyl-derived C^{14} which has been incorporated into the hot-water-soluble or dilute-alkali-soluble portions of the coleoptile wall is slowly lost again when the tissue is incubated in unlabeled methionine. This decorporation of methyl-derived carbon is slowed in the presence of IAA. Similar decorporation of glucose carbon is depressed by IAA.

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† Postdoctoral Fellow, National Science Foundation.

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THE PERMEABILITY OF POTATO TISSUE TO WATER

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Introduction.—Of the possible mechanisms whereby auxin causes plant cells to enlarge, the two which have not been eliminated are, in essence, (*a*) decrease of the mechanical coherence of the cell wall by a metabolic process, resulting in the entry of water under osmotic forces, and (*b*) increased uptake and accumulation of water by a metabolic process, resulting in the mechanical stretching or expansion of the cell wall. In both instances metabolism is also used to redeposit cell-wall constituents and thus to maintain the wall's integrity and thickness. The two alternatives have been described schematically and the evidence for and against them set out.[1, 2] Although there are few critical data distinguishing between the two, the second alternative, often referred to as "nonosmotic water uptake" or a "metabolic water pump," has attracted more attention in recent years. Another explanation sometimes offered, based on an increase of the "permeability" of the cell to water, is closely related to it.

The availability of tritiated water, T_2O or THO, makes possible a fresh approach to this problem, since one can directly follow the entry of radioactive water into a tissue, or its exit therefrom. Water permeability can thus be measured under normal conditions and without subjection to plasmolysis or any other change. The influence of auxin or other factors on the entry can be readily studied.

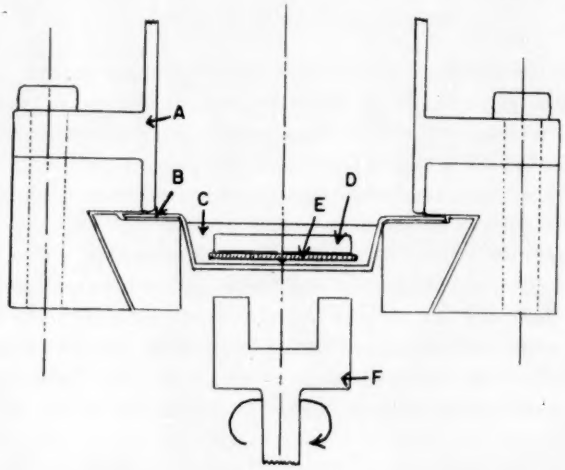
A somewhat similar approach, using deuterium water, followed by extraction, purification, and determination by densitometric methods has been made by Buffel,[3] by Ketellapper,[4] and recently, using mass-spectrometric methods, by Ordin and Bonner.[5]

Procedure.—The range of the beta particle from tritium is so short that T_2O is detectable only at the extreme surface of a liquid. For this reason the tissue was allowed to take up T_2O beforehand, and the subsequent rate of outflow of the T_2O into H_2O was followed. As material, disks of potato tuber were selected because the rate and duration of water uptake in them has been studied in detail.[6] The

process is aerobic, sensitive to various enzyme inhibitors, and promoted by auxins in low concentration.

The disks, 1.5 cm. in diameter and of various determined thicknesses from 0.[illegible] to 4.5 mm., were cut with a hand microtome from Maine potatoes (var. Katahdin). They were washed in running tap water for 25–30 minutes and then allowed to stand for 24 hours in a flat shallow dish of distilled water to complete the washing and to reach full turgor. The disks were then suspended on nets in Petri dishes as previously described,[6] one side of each disk being exposed to the air and the other to the medium.

After an uptake period of 2–6 days at 25° C. in the dark with or without auxin, in 8 or 16 microcuries/cc. T_2O, the disks were blotted in a standard manner[6] and weighed, and one of them was then transferred to a shallow aluminum planchette (2 cm. in diameter, 6 mm. deep), in which it rested on a flat steel disk and was submerged in 1 cc. of distilled water. The planchette was introduced into a gas flow counter (95 per cent A, 5 per cent CO_2) which was held at 30° C. to minimize condensation. Below this was a motor-driven magnet which rotated the steel disk (Fig. 1). Thus the tissue and the solution were in constant movement, allowing



CROSS SECTION OF COUNTING APPARATUS X2

FIG. 1.—Arrangement for following outflow of T_2O. *A*, brass counting tube; *B*, aluminum planchette; *C*, 1 ml. of water; *D*, potato disk; *E*, steel spinner; *F*, motor-driven permanent magnet.

rapid equilibration with one another without breaking the surface of the solution or disturbing the geometry in any way. The only disadvantage was that the sections were temporarily submerged, but, since the counting period was so brief, no damage to the tissue could have resulted. The counter was connected to a scaler and thence to a count-rate meter whose output was continuously recorded. In the resulting curve the background variations were smoothed, and the time to reach the half-maximum value (that is, half-maximum T_2O concentration in the H_2O) was determined.

Results.—Typical outflow curves are shown in Figure 2. The shape of the curve is that to be expected from a simple two-compartment diffusion system. Equilibrium is reached within about 20 minutes at room temperature, which means that the T_2O and H_2O have completed their exchange, down to the center of the tissue section, within this time. The time for half-maximum value is 1.5 minutes.

Sections which had grown for 6 days in auxin (indoleacetic acid, 10 mg. per liter) appeared to reach equilibrium in a shorter time, but the difference shown in Figure 2 is too small to be significant. In order to study the difference more thoroughly, thicker sections were cut. These require a longer time to reach equilibrium and

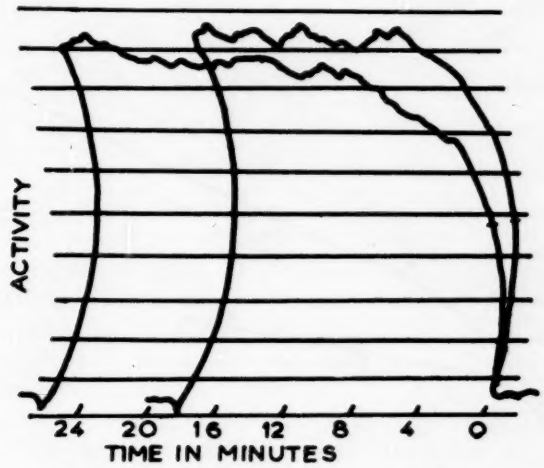


FIG. 2.—Change of radioactivity of the external water. *Lower curve:* section kept in water; *upper curve:* section kept in auxin. Sections 1 mm. thick.

hence allow more precise measurement. For sections of more than 1 mm. thickness, Figure 3 shows clearly that the time for half-maximum value is reached much more rapidly by the sections which have grown in auxin. At 2 mm. the difference is about 50 per cent, though the scatter of the values does not permit a precise evaluation of the ratio.

A curious feature of these experiments is the observation that when the outflow of T_2O is measured at 3° C., it is considerably slower than at 30° C. As a mean of many observations at different disk thicknesses, the ratio of times for half-maximum value at 3° and at 30° C. is 1.56. Since there is no reason to involve any metabolic component, it is probable that this difference is due to the change of viscosity of water. At 3° C. this is 1.62 centipoises, and at 30° C. it is 0.80, a ratio of 2, which is ample to account for the temperature effect on T_2O outflow.

Discussion.—The very rapid exchange of T_2O between the cells and the solution makes any active, metabolism-dependent water-pumping system exceedingly unlikely. The expenditure of energy required to take up or to retain water against so "leaky" a membrane would appear prohibitive. Indeed, it has been claimed (using data for the permeability of water derived from plasmolysis rates) that metabolic water uptake cannot take place because insufficient energy is available.[7]

These findings support Levitt's calculations. Ordin and Bonner[5] have reached similar conclusions.

More crucial is the fact that if auxin enabled the cells to take up or to retain water, some slightly retarded outflow in auxin-treated cells would be expected. On the contrary, the sections in auxin showed a clear increase in outflow rate. Since the time in auxin had been long, the increased rate is probably the result of growth and indicates that the newly formed cell wall or membrane is thinner or of a more open texture than that originally present. In any event, taking the present results along with previous growth studies, especially those using osmotic inhibition,[2] it is clear that the former of the two alternatives stated at the outset provides the mechanism of cell enlargement and therefore the seat of auxin action.

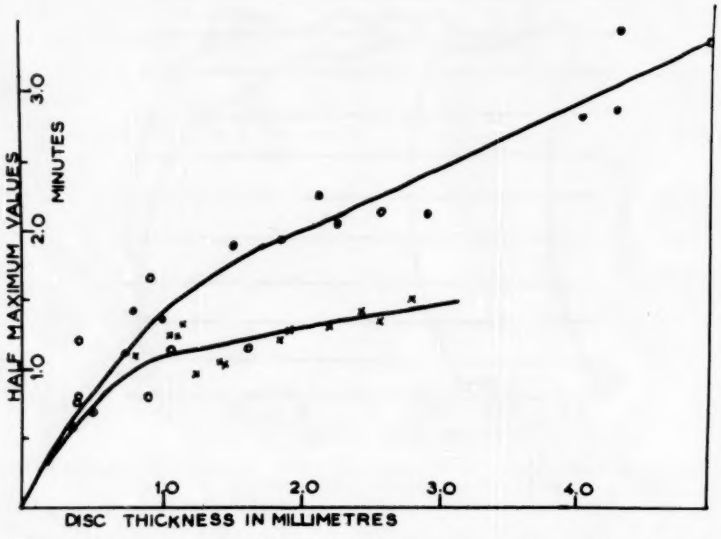


FIG. 3.—Time for half-maximum radioactivity of the external water as a function of disk thickness. *Lower curve:* sections kept in auxin (10 mg. per liter of indoleacetic acid); *upper curve:* sections kept in water.

Summary.—Potato tuber disks such as have been used extensively for water-uptake studies were allowed to take up tritium water, T_2O, and their equilibration with H_2O was then followed. The T_2O in the disks reached equilibrium with the external H_2O within about 20 minutes, the time for half the maximum value being about 1.5 minutes. If there was previous exposure to indoleacetic acid, the equilibrium was reached even more rapidly, which is the reverse of what would be predicted if a metabolic water uptake were being promoted. The process is slowed about 50 per cent by cooling to 3° C. The findings are extended and supported by the use of disks of varying thicknesses. It is concluded that uptake or retention of water by a direct metabolic reaction followed by modification of the cell wall cannot be the mechanism of growth or of auxin action. Hence the auxin action must be exerted directly upon the properties of the cell wall.

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THE ION-PAIR EQUILIBRIUM OF ELECTROLYTE SOLUTIONS IN DIOXANE-WATER MIXTURES

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Bjerrum[1] has developed a theory of ion-pair equilibrium in electrolyte solutions which relates the dissociation constant of this equilibrium with the dielectric constant of the solvent medium. Fuoss and Kraus[2] have carried out measurements with solutions of tetra*iso*amylammonium nitrate in dioxane-water mixtures which conform rather satisfactorily with this theory. Also, Martel and Kraus[3] have recently reported measurements with several salts in dioxane-water mixtures covering the range from pure water, where no ion association occurs, to mixtures in which association sets in and increases as the dielectric constant decreases.

The measurements of Fuoss and Kraus, with one exception at $D = 38$, relate to mixtures with dielectric constants ranging from 12 down. How closely the results at higher dielectric constants are reproduced by Bjerrum's theory cannot be determined from the one measurement at high dielectric constant. While the measurements of Martel and Kraus covered a range both above and below the critical dielectric constant, above which no ion association occurs, the number of mixtures measured in the lower dielectric-constant range, where ion association occurs, was not sufficiently large to permit precise determination of how well Bjerrum's theory reproduces the observed values.

Accordingly, it seemed worth while to carry out measurements with several salts in dioxane-water mixtures from pure water to the lowest dielectric constant at which precise measurements could be carried out by means of the techniques employed. Measurements were carried out with solutions of tetrabutylammonium bromide and tetramethylammonium picrate at relatively short dielectric-constant intervals below the critical dielectric constant.

The techniques and procedures employed were the same as those used and described by Martel and Kraus[3] as well as by others[4] in earlier papers. It seems unnecessary to repeat these descriptions.

The results of the measurements are presented in Tables 1 and 2. The composition of the mixture and its dielectric constant appear at the head of each subtable. In the first column is given the concentration in moles of solute per liter of

TABLE 1

CONDUCTANCE OF Bu_4NBr IN DIOXANE-WATER MIXTURES AT 25°

$C \times 10^4$	Λ	Λ_0'	$C \times 10^4$	Λ	Λ_0'
A. 0 Per Cent Dioxane, D = 78.48			B. 10 Per Cent Dioxane, D = 70.33		
1.36596	96.489	97.458	1.53596	79.852	80.781
2.70109	96.069	97.432	3.07132	79.458	80.771
5.39813	95.478	97.405	6.06456	78.909	80.753
10.7507	94.628	97.346	12.0968	78.110	80.715
21.4209	93.391	97.228	24.0982	76.945	80.620
C. 20 Per Cent Dioxane, D = 61.86			D. 30 Per Cent Dioxane, D = 53.28		
1.26587	66.753	67.532	1.54373	56.402	57.233
2.58724	66.438	67.553	3.07621	56.059	57.234
5.19920	65.966	67.547	6.15221	55.564	57.223
10.3575	65.299	67.530	12.1923	54.865	57.202
20.6801	64.331	67.482	24.3027	53.871	57.169
E. 35 Per Cent Dioxane, D = 48.91			F. 40 Per Cent Dioxane, D = 44.54		
1.52562	52.235	53.063	1.55249	48.561	49.408
3.06130	51.883	53.055	3.07953	48.199	49.393
6.12415	51.372	53.030	6.15139	47.677	49.364
12.2253	50.654	52.997	12.2773	46.946	49.327
24.3999	49.683	52.992	24.4890	45.929	49.292
G. 45 Per Cent Dioxane, D = 40.20			H. 50 Per Cent Dioxane, D = 35.85		
1.53489	45.384	46.252	1.81874	42.634	43.633
3.05159	45.009	46.232	3.68305	42.177	43.598
6.04577	44.474	46.196	7.43318	41.514	43.532
12.0267	43.719	46.147	14.7885	40.617	43.462
23.9273	42.698	46.122	29.3970	39.402	43.410
I. 55 Per Cent Dioxane, D = 31.53			J. 60 Per Cent Dioxane, D = 27.21		
1.73541	40.382	41.445	1.50351	38.503	39.616
3.49580	39.882	41.389	2.99216	37.953	39.520
7.00742	39.154	41.285	5.94550	37.176	39.382
13.9217	38.175	41.174	11.8443	36.101	39.208
27.9230	36.848	41.091	23.6576	34.688	39.072
K. 65 Per Cent Dioxane, D = 23.14			L. 70 Per Cent Dioxane, D = 19.07		
1.78996	36.615	38.016	1.61708	34.915	36.526
3.54409	35.865	37.832	3.24167	33.868	36.135
7.00919	34.796	37.550	6.48920	32.411	35.592
13.9486	33.375	37.244	12.9556	30.606	35.064
27.8049	31.581	37.028	25.8160	28.446	34.704

TABLE 2

CONDUCTANCE OF Me_4NPi IN DIOXANE-WATER MIXTURES AT 25°

$C \times 10^4$	Λ	Λ_0'	$C \times 10^4$	Λ	Λ_0'
A. 0 Per Cent Dioxane, D = 78.48			B. 10 Per Cent Dioxane, D = 70.33		
1.50350	74.160	75.114	1.67319	61.631	62.536
2.99877	73.780	75.127	3.31610	61.298	62.573
5.96982	73.265	75.167	6.58131	60.810	62.606
11.9003	72.561	75.247	13.1967	60.164	62.708
23.6842	71.606	75.396	26.3864	59.284	62.884
C. 20 Per Cent Dioxane, D = 61.86			D. 30 Per Cent Dioxane, D = 53.28		
1.49638	51.774	52.562	1.73762	43.882	44.696
3.02120	51.456	52.575	3.44195	43.606	44.753
6.00297	51.036	52.614	6.84819	43.152	44.779
11.9937	50.467	52.699	13.6662	42.581	44.868
23.9481	49.700	52.856	27.2737	41.842	45.078
E. 35 Per Cent Dioxane, D = 48.91			F. 40 Per Cent Dioxane, D = 44.54		
1.60239	40.714	41.495	1.85842	37.898	38.747
3.20754	40.448	41.529	3.72996	37.566	38.769
6.41841	40.001	41.563	7.45965	37.121	38.823
12.7995	39.455	41.664	14.8980	36.548	38.957
25.5934	38.809	41.938	29.7662	35.805	39.218

$C \times 10^4$	Λ	Λ_0'
G. 45 Per Cent Dioxane, D = 40.20		
1.63426	35.751	36.569
3.28837	35.343	36.504
6.56446	34.911	36.552
13.1072	34.353	36.674
26.2044	33.633	36.921
I. 55 Per Cent Dioxane, D = 31.53		
1.85315	32.333	33.331
3.70072	31.951	33.361
7.35544	31.434	33.425
14.7007	30.776	33.596
29.3854	29.943	33.947
K. 62.5 Per Cent Dioxane, D = 25.15		
0.78181	31.298	32.081
1.56973	30.982	32.093
3.13939	30.533	32.104
6.27126	29.941	32.163
12.5279	29.138	32.274
M. 67.5 Per Cent Dioxane, D = 21.12		
1.21859	30.521	31.685
2.83036	29.877	31.651
6.06563	29.067	31.663
12.5146	28.035	31.772
25.4039	26.804	32.160

$C \times 10^4$	Λ	Λ_0'
H. 50 Per Cent Dioxane, D = 35.85		
1.64103	33.778	34.641
3.26646	33.449	34.668
6.51083	33.022	34.744
12.9908	32.441	34.877
25.9693	31.702	35.157
J. 60 Per Cent Dioxane, D = 27.21		
1.58338	31.371	32.411
3.16711	30.957	32.428
6.32921	30.416	32.498
12.6188	29.699	32.643
25.1790	28.795	32.974
L. 65 Per Cent Dioxane, D = 23.14		
1.67840	30.629	31.872
3.36779	30.103	31.863
6.72295	29.408	31.896
13.4462	28.515	32.041
26.8340	27.455	32.467
N. 70 Per Cent Dioxane, D = 19.07		
1.85010	30.026	31.621
3.69980	29.275	31.528
7.36197	28.321	31.496
14.6807	27.122	31.615
29.3102	25.727	32.129

solution, in the second column the equivalent conductance Λ, and in the third column Λ_0', which is defined by the following expression:

$$\Lambda_0' \equiv \frac{\Lambda + \beta\sqrt{C}}{1 - \alpha\sqrt{C}}. \tag{1}$$

The difference $\Lambda_0' - \Lambda_0$ yields the deviation from the simple Onsager equation within the limits of experimental error at low concentrations.

All measurements were carried out at 25° ± 0.001°. Dielectric constants were interpolated from the data of Critchfield, Gibson, and Hall[5] and viscosities from the data of Geddes,[6] after correcting for the latest values for the viscosity of water.[7]

To evaluate the dissociation constant K of the electrolyte in these mixtures, we have employed the method of Martel and Kraus.[3] At low concentrations the deviation from Onsager's simple equation, in Λ units, is given by the equation

$$\Lambda_0' - \Lambda_0 = BC, \tag{2}$$

where B is an empirical constant. In dilute solutions, up to about 2×10^{-3} N, the deviation is a linear function of C.

As the dioxane content of the solvent increases, the viscosity increases and Λ_0 decreases. Accordingly, we normalize equation (2) by dividing through by Λ_0, which leads to

$$\frac{\Lambda_0'}{\Lambda_0} - 1 = B'C, \tag{3}$$

where

$$B' = \frac{B}{\Lambda_0}. \tag{4}$$

If ion association occurs, the conductance decrease, $\Delta_a\Lambda$, due to association is very nearly a linear function of C, and we may write

$$\Delta_a\Lambda = B_aC, \tag{5}$$

and for the total deviation from Onsager's equation we have

$$\Lambda_0' - \Lambda_0 = (B + B_a)C = B^*C, \tag{6}$$

where

$$B^* = B + B_a. \tag{7}$$

Thus we also have

$$\frac{\Lambda_0'}{\Lambda_0} - 1 = (B' + B_a')C = B'^*C. \tag{8}$$

If the value of B is known for an electrolyte in a given mixture, we may subtract the deviation BC from the observed conductance, and we obtain

$$\Lambda - BC = \Lambda^* = \Lambda_0 - (\alpha\Lambda_0 + B)\sqrt{C}; \tag{9}$$

Λ^* conforms to Onsager's equation, and K may be evaluated by the method of Fuoss.[8]

As it happens, the value of B' varies very nearly as a linear function of the dielectric constant, D, and its change is often very small. Accordingly, the value of B' for mixtures of lower dielectric constant, where association occurs, may be found by extrapolating to lower values of D. The value of B is then found by simply multiplying B' by Λ_0. Thus reliable values of K may be found by means of equation (9).

In Tables 3A and 3B are shown values of B and B' for Bu_4NBr and Me_4NPi, respectively. Values of B^* and B'^* relate to mixtures in which ion association occurs. Values of Λ_0 are shown in the second column and values of the dielectric constant in the first column.

TABLE 3A

VALUES OF Λ_0, B, AND B' FOR Bu_4NBr IN DIOXANE-WATER MIXTURES AT 25°

D	Λ_0	B	B'	D	Λ_0	B	B'
78.48	97.464	−111	−1.1	40.20	46.272	− 125*	− 2.70*
70.33	80.792	− 64	−0.79	35.85	43.668	− 186*	− 4.26*
61.86	67.560	− 30	−0.44	31.53	41.497	− 309*	− 7.4*
53.28	57.245	− 33*	−0.58*	27.21	39.704	− 610*	−15.4*
48.91	53.074	− 62*	−1.17*	23.14	38.241	−1,500*	−39.2*
44.54	49.422	− 91*	−1.84*	19.07	37.010	−2,950*	−80.*

TABLE 3B

VALUES OF Λ_0, B, AND B' FOR Me_4NPi IN DIOXANE-WATER MIXTURES AT 25°

D	Λ_0	B	B'	D	Λ_0	B	B'
78.48	75.091	+128	+1.7	35.85	34.621	+125	+ 3.6
70.33	62.520	+141	+2.3	31.53	33.307	+130	+ 3.9
61.86	52.533	+136	+2.6	27.21	32.400	+ 87*	+ 2.7*
53.28	44.672	+144	+3.2	25.15	32.087	+ 38*	+ 1.2*
48.91	41.475	+137	+3.3	23.14	31.893	−142*	− 4.5*
44.54	38.727	+136	+3.5	21.12	31.723	−254*	− 8.0*
40.20	36.470	+122	+3.3	19.07	31.693	−528*	−17.2*

In Figure 1 are shown values of B' and B'^* as a function of dielectric constant. It will be noted that at higher values of D both plots are linear within the limit of experimental error, and they change but little as D decreases.

For Bu_4NBr the plot begins to fall away at a dielectric constant of 53.3 and for Me_4NPi at a dielectric constant of 27.2. Thereafter, values of B'^* fall off increasingly due to ion association. To find the value of B for the purpose of evaluating K, the linear portions of the plots are extrapolated to the appropriate value of D. It will be noted that B' is negative for Bu_4NBr in water and that the negative values decrease as D decreases, until D reaches a value where ion association sets in ($D = 48.9$). For Me_4NPi, B' is positive and increases as D decreases, until ion association occurs.

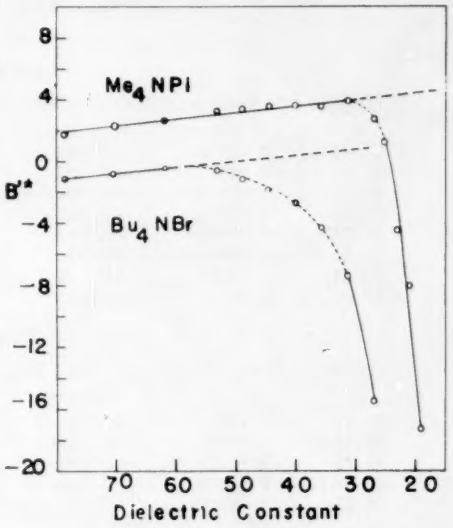

FIG. 1.—Plots of B'^* versus D

In Table 4 are given values of K over the composition range in which ion association occurs. Values of K appear in the second column and values of B, as employed in the calculations, in the third column. It will be noted that, for the bromide, B becomes positive for $D < 48.91$.

TABLE 4

VALUES OF THE DISSOCIATION CONSTANTS, K, OF SOME SALTS IN DIOXANE-WATER MIXTURES AT 25°

A. Tetrabutylammonium Bromide			B. Tetramethylammonium Picrate		
D	K	B	D	K	B
48.91	0.77	0	27.21	0.33	+135
44.54	.46	+ 7	25.15	.27	+136
40.20	.28	+13	23.14	.098	+138
35.85	.18	+20	21.12	.049	+140
31.53	.10	+26	19.07	0.038	+143
27.21	.049	+31			
23.14	.023	+34			
19.07	0.0086	+41			

Ion association sets in at a much higher dielectric constant with the bromide than with the picrate. The former is markedly associated at $D = 48.9$, while the latter salt begins to associate at $D = 27.2$. At this value of D, $K = 0.33$ for the picrate and 0.049 for the bromide. The picrate is a much stronger electrolyte than the bromide.

It is of interest to note that the dissociation constants of Me_4NPi and Bu_4NBr in a mixture of $D = 19.1$ are, respectively, 38×10^{-3} and 8.6×10^{-3}. In acetone[9] of $D = 20.5$, they are, respectively, 11.2×10^{-3} and 3.3×10^{-3}. The dissociation constant of an electrolyte is dependent on the nature of the solvent as well as upon its dielectric constant and the temperature.

In Tables 5A and 5B are given numerical values of quantities relating to the calculations. The third column of each table gives values of the linear deviation correction, BC; the fourth column, values of $\Delta_a\Lambda$, the conductance decrease due to association; and the fifth column, values of the degree of association in per cent, $100(1 - \gamma)$.

TABLE 5A

DEGREE OF ASSOCIATION OF Bu_4NBr IN DIOXANE-WATER MIXTURES AT 25°

D	$C \times 10^4$	BC	$\Delta_a\Lambda$	$100(1 - \gamma)$	f
48.91	3.06130	−0.001	−0.019	0.033	0.959
44.54	3.07953	+0.002	−0.030	0.064	0.952
40.20	3.05159	+0.004	−0.042	0.095	0.946
35.85	3.68305	+0.007	−0.076	0.182	0.930
31.53	3.49580	+0.009	−0.117	0.295	0.918
27.21	2.99216	+0.009	−0.192	0.506	0.906
23.14	3.54409	+0.012	−0.543	1.08	0.872
19.07	3.24167	+0.013	−0.970	2.74	0.840

TABLE 5B

DEGREE OF ASSOCIATION OF Me_4NPi IN DIOXANE-WATER MIXTURES AT 25°

D	$C \times 10^4$	BC	$\Delta_a\Lambda$	$100(1 - \gamma)$	f
27.21	3.16711	+0.043	−0.016	0.052	0.903
25.15	1.56973	+0.021	−0.015	0.052	0.922
23.14	3.36779	+0.046	−0.094	0.262	0.874
21.12	2.83036	+0.040	−0.112	0.381	0.868
19.07	3.69980	+0.054	−0.248	0.754	0.829

For Bu_4NBr, the linear correction term amounts to, at most, 10 per cent of the conductance decrease, $\Delta_a\Lambda$, due to association. For solutions in mixtures of lowest dielectric constant, this correction term becomes almost negligible. For Me_4NPi, however, the correction term is relatively large in comparison with $\Delta_a\Lambda$. For $D = 27.2$, $BC = 0.043$ and $\Delta_a\Lambda = -0.016$; for $D = 19.07$, $BC = 0.054$ and $\Delta_a\Lambda = -0.248$; even at the lowest value of D the linear deviation term is significant.

The degree of association, $1 - \gamma$, may be obtained directly from the conductance decrease, $\Delta_a\Lambda$, without evaluation of K, for

$$1 - \gamma = \frac{\Delta_a\Lambda}{\Lambda}.$$

However, it is preferable to evaluate K by the Fuoss procedure since this seems to average the value of individual measurements.

In Figure 2 are shown plots of the degree of association, $100(1 - \gamma)$, for the two salts as a function of dielectric constant. It will be noted that, once association has set in, it increases much more rapidly for Me_4NPi than it does for Bu_4NBr. This was to be expected, since the percentage change in D near the critical value is much greater for the picrate than for the bromide.

In the sixth columns of Tables 5A and 5B are given values of the activity coefficient, f, for the different mixtures. In mixtures of lower dielectric constant, the value of f departs markedly from unity.

Since

$$1 - \gamma = \frac{C\gamma^2 f^2}{K}, \tag{10}$$

it is apparent that $\Delta_a\Lambda$ will not be strictly a linear function of C.

It is of interest to see how closely Bjerrum's theory reproduces the experimental values. Bjerrum has derived an equation which relates the constant K with the product DT through a parameter a, which may be, and sometimes is, interpreted as the distance between the centers of charge of the ions in the ion pairs. From this equation it is also possible to calculate the critical dielectric constant, D_c, above which ion association does not occur.

In the third column of Table 6 are shown values of the parameter a; in the fourth column, values of D_c, as calculated from the K values of the second column.

TABLE 6

VALUES OF a AND D_c FOR SOME SALTS IN DIOXANE-WATER MIXTURES AT 25°

A. TETRABUTYLAMMONIUM BROMIDE				B. TETRAMETHYLAMMONIUM PICRATE			
D	K	a (in A)	D_c	D	K	a (in A)	D_c
48.91	0.77	5.09	55.0	27.21	0.33	10.1	27.7
44.54	.46	5.34	52.5	25.15	.27	11.0	25.5
40.20	.28	5.74	48.8	23.14	.098	11.2	25.0
35.85	.18	6.18	45.3	21.12	.049	11.4	24.6
31.53	.10	6.68	41.9	19.07	0.038	12.7	22.1
27.21	.049	6.96	40.3				
23.14	.023	7.15	39.2				
19.07	0.0086	6.77	41.4				

It will be noted that the parameter a is not a constant. For Bu_4NBr, a increases from 5.09 A at $D = 48.9$ to 7.15 A at $D = 23.1$. At $D = 19.07$, the value of a falls to 6.77 A. This lower value may be valid, or it may be in error due to experimental error.

The value of D_c for Bu_4NBr, as computed from the values of a, varies regularly and widely, namely, from 39.2 at $D = 23.1$ to 55.0 at $D = 48.9$. There is no question but that the salt is associated for all mixtures from $D = 48.9$ down. In all likelihood, D_c is not far from 55.

For Me_4NPi, the variation of a is much smaller than it is for the bromide. From $D = 27.2$ to $D = 19.07$, a increases from 10.1 A to 12.7 A. The variation of D_c is also much smaller; the value of D_c calculated from a at $D = 27.2$ is 27.7, only a little above that of the solvent mixture measured. If one assumes D_c to be 31.5 and computes the value of a from Bjerrum's equation, one obtains a value of 8.78 A. Since $a = 10.1$ A at $D = 27.2$, it is very unlikely that the electrolyte is associated at $D = 31.5$.

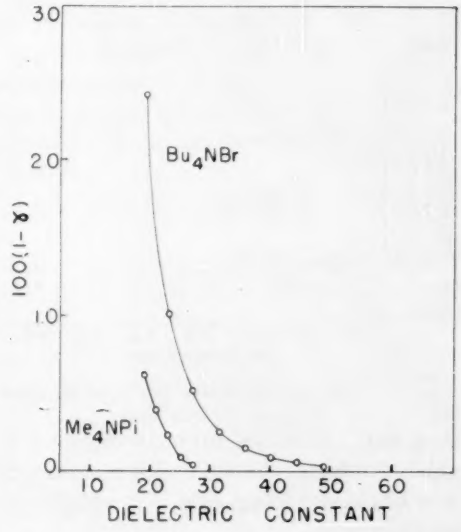

FIG. 2.—Plots of degree of association versus D

The conductance of an ion in a medium depends on the viscosity of the medium and, in general, decreases as the viscosity increases. For large ions the conductance

is approximately inversely proportional to the viscosity, in accordance with Walden's rule. If Walden's rule holds, the conductance-viscosity product, $\Lambda_0\eta$, should have a constant value. Unfortunately, ion conductances are not generally known, and the conductance which we measure is the sum of the conductances of the two ions. Even so, if both the ions are large, the conductance-viscosity product of the electrolyte is approximately a constant. There are very few measurements in which values of Λ_0 are known over a rather wide viscosity range in similar solvents. However, these dioxane-water mixtures provide a convenient means of investigating the dependence of conductance on viscosity, for the viscosity change as we proceed from water to mixtures of high dioxane content is rather large.

In Figure 3 are shown values of the conductance-viscosity product, $\Lambda_0\eta$, for tetramethylammonium picrate and tetrabutylammonium bromide as determined in the present investigation. On the same figure are shown values for tetrabutylammonium iodide as determined by Martel and Kraus.[3] It will be noted that the plots for tetramethylammonium picrate and tetrabutylammonium bromide are very similar. Initially, and up to a mixture containing 30 per cent or more of dioxane, the value of $\Lambda_0\eta$ changes but little; thereafter, it decreases rather sharply at a dioxane content above 50 per cent.

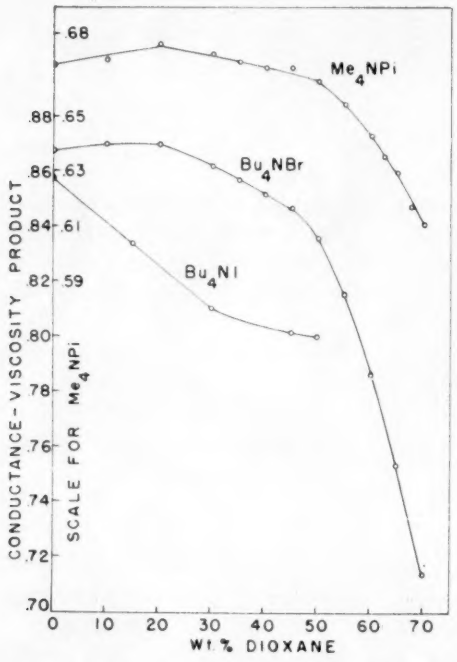

FIG. 3.—Plots of $\Lambda_0\eta$ versus per cent dioxane

The plot for tetrabutylammonium iodide is very different from that of the corresponding bromide. The value of the product falls off rather sharply at the start and levels off as the composition approaches 50 per cent dioxane.

Now the positive ion in the case of these two halides is the same, and the differences observed in the course of $\Lambda_0\eta$ values can be due only to the difference in the behavior of the bromide and iodide ions. Initially, the bromide ion is slowed up in proportion to the viscosity increase on addition of dioxane, but it is slowed up relatively greatly at high concentrations of dioxane. On the other hand, the iodide ion is slowed up markedly on the initial addition of dioxane and is slowed up only in proportion to viscosity increase at high concentrations of dioxane.

No far-reaching conclusion can be drawn from the limited data available at this time, but it is apparent that much valuable information might be obtained by carrying out measurements in mixtures whose viscosities change greatly with composition.

* The investigation here reported was supported by the Office of Naval Research under Contract N7onr 35809.

† This paper is based on a portion of a thesis presented by Philip L. Mercier in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Brown University, June, 1955.

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ON THE CHEMICAL STRUCTURE OF THE REACTIVE SITES OF ANTIBODY MOLECULES

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Quantitative evidence has been obtained by us that a single ionized carboxyl group is critically involved in the formation of the antigen-antibody bond in both the rabbit anti–bovine serum albumin[1] and the anti-ovalbumin[2] systems. This information was derived from ultracentrifugal studies of the effect of acid pH on antigen (Ag)–antibody (Ab) equilibria. As a consequence, and because of other as yet qualitative information, it was suggested that a single positively charged group is also present on the complementary site in the Ag-Ab bond. It is not possible, however, to decide from these results which group is associated with the Ag, and which with the Ab, molecule. In this paper are reported chemical studies which strongly indicate that amino groups are involved in the reactive sites of the antibody to bovine serum albumin (BSA).

We have found that BSA which is extensively modified at its free amino groups (ϵ-NH_2 and terminal α-NH_2), either by conversion to guanidino groups or by acetylation, retains most of its ability to precipitate antibodies prepared against unmodified BSA. This confirms the findings of Ehrenpreis and Maurer.[3] Furthermore, however, acetylation of anti-BSA antibody causes it to lose its ability to precipitate BSA. That this inactivation of Ab is a specific chemical effect, as opposed to nonspecific denaturation, is demonstrated by the fact that Ab retains most of its activity upon acetylation in the presence of a large excess of BSA, clearly because of the protection of the Ab reactive sites by Ag-Ab bond formation.[4]

EXPERIMENTAL METHODS

a) Proteins.—BSA and rabbit antisera to BSA were similar to materials previously employed.[1] The γ-globulin fraction of the antisera, containing 27 per cent anti-BSA antibodies, was precipitated with $^1/_3$ saturated $(NH_4)_2SO_4$, and after two centrifugings and washings was dissolved in, and dialyzed against, phosphate buffer (pH, 7.5; $\Gamma/2$, 0.1). A solution of BSA–anti-BSA complexes containing about

84 per cent BSA and 16 per cent Ab by weight was prepared as previously,[1] by dissolving a specific precipitate in excess BSA.

b) Guanidination of BSA.—This was performed in a manner similar to that of Hughes *et al.*[5] To 0.410 gm. of *o*-methyl isouronium sulfate[6] in H_2O, enough 1.0 *M* KOH was added to give a solution of pH 10.7 and total volume 10 ml. After cooling the solution to 0° C., it was added to 1.0 gm. of BSA and kept at 0° C. for 3 days. It was then brought to pH 8.0 with the careful addition of glacial acetic acid at 0° C. and was then dialyzed against phosphate buffer (pH, 7.5; $\Gamma/2$, 0.1).

c) Acetylation.—The method of Fraenkel-Conrat *et al.*[7] was used. Six solutions were acetylated: (1) 35 mg. BSA in 1.5 ml. 1 per cent NaCl; (2) 26 mg. inert γ-globulin and 9 mg. anti-BSA Ab in 1.5 ml. phosphate buffer (pH, 7.5; $\Gamma/2$, 0.1); (3) 29 mg. inert γ-globulin, 10 mg. anti-BSA Ab, and 40 mg. ovalbumin in 2 ml. phosphate buffer; (4) 300 mg. BSA and 60 mg. anti-BSA Ab in 15 ml. phosphate buffer; (5) 104 mg. BSA in 3 ml. phosphate buffer; and (6) 88 mg. inert γ-globulin and 32 mg. anti-BSA Ab in 4.4 ml. phosphate buffer. To each solution an equal volume of saturated sodium acetate was added, and the whole brought to 0° C. With stirring at 0° C, 0.1 ml. C.P. acetic anhydride was added for each 40 mg. of protein, the total addition in each experiment taking 1 hour. The additions were made gradually from microburettes. Solutions 1,2, and 3 were then dialyzed against phosphate buffer (pH, 7.5; $\Gamma/2$, 0.1), and solutions 4, 5, and 6 against veronal buffer (pH, 8.68; $\Gamma/2$, 0.1).

d) Electrophoresis and Ultracentrifugation.—Electrophoresis was performed in a Perkin-Elmer Model 38 Tiselius apparatus, in veronal buffer (pH, 8.68; $\Gamma/2$, 0.1), for 4,500 sec. at 7.0 milliamp. Ultracentrifuge runs with the same solutions were carried out in a Spinco Model E instrument at 59,780 rpm.

EXPERIMENTAL RESULTS

a) Reactivity of Guanidinated and Acetylated BSA.—Parallel Ag-Ab titrations were performed with guanidinated BSA (GuBSA), acetylated BSA (AcBSA, solution 1), and unmodified BSA, against the same dilution of a γ-globulin solution containing anti-BSA. Dilutions of the antigens were made from stock solutions whose protein concentrations were determined by differential refractometry.[8] At the equivalence zone, 0.47, 0.41, and 0.48 mg. of protein were precipitated by the addition of 0.063 mg. of GuBSA, AcBSA, and BSA, respectively, to 1 ml. of the anti-BSA solution. These results are in accord with the more extensive studies already mentioned.[3]

b) Reactivity of Acetylated Anti-BSA.—Solutions 2, 3, and 6 were tested for Ab activity against BSA. All showed a drastic loss of activity. For example, when parallel titrations were performed with solution 2 and its unacetylated control, the former at a total protein concentration seven fold greater than the latter, no detectable precipitates (as determined by Nessler nitrogen analyses) formed at any BSA dilution, whereas a maximum of 0.48 mg. of protein was precipitated by 0.063 mg. of BSA. This demonstrates that less than 1 per cent of the original Ab activity remained. Similar results were obtained with solutions 3 and 6, but with the latter a faint ring test was obtained against a solution containing 1 mg. of BSA/ml, indicating that some (less than 1 per cent) of the activity was retained.

c) Protection of Ab against Acetylation by Combination with BSA.—In order to demonstrate that the anti-BSA antibody retains its activity when it is acetylated in the presence of an excess of BSA, it is sufficient to perform electrophoresis and ultracentrifuge experiments on the resultant solution. Should Ag-Ab complexes, as identified by their electrophoretic mobilities and sedimentation velocities, persist, then the Ab is still active. If, on the other hand, the Ab has been inactivated, the electrophoresis and ultracentrifuge patterns should correspond to those of a mixture of separately acetylated BSA and inert rabbit γ-globulin. Therefore, a solution of acetylated BSA–anti-BSA complexes (solution 4), the same solution prior to acetylation, and a mixture prepared from solutions 5 and 6, containing 84 per cent acetylated BSA and 16 per cent acetylated γ-globulin, were subjected to electrophoresis and ultracentrifugation in veronal buffer (pH, 8.68; Γ/2, 0.1). The electrophoresis patterns of the acetylated complexes (Fig. 1, *a*) clearly reveal the

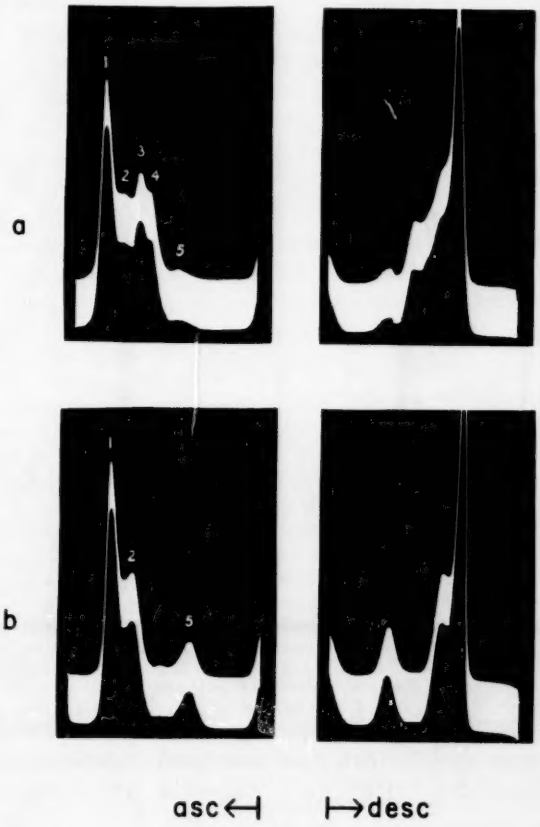

FIG. 1.—Electrophoresis patterns of (*a*) acetylated BSA–anti-BSA complexes and (*b*) a mixture of separately acetylated BSA and rabbit γ-globulin, in veronal buffer (pH, 8.68, Γ/2, 0.1). Ascending and descending starting positions are indicated by the arrows. Boundaries *1* and *2* are due to acetylated BSA, boundaries *3* and *4* to Ag-Ab complexes, and boundary *5* to acetylated γ-globulin.

presence of Ag-Ab complexes (boundaries *3* and *4*) not present in the mixture of separately acetylated BSA and γ-globulin[9] (Fig. 1, *b*). Similarly, the ultracentri-

fuge patterns of the solution of acetylated complexes (Fig. 2, *b*) demonstrate the persistence of Ag-Ab complexes[8] present in the unacetylated solution (Fig. 2, *a*)

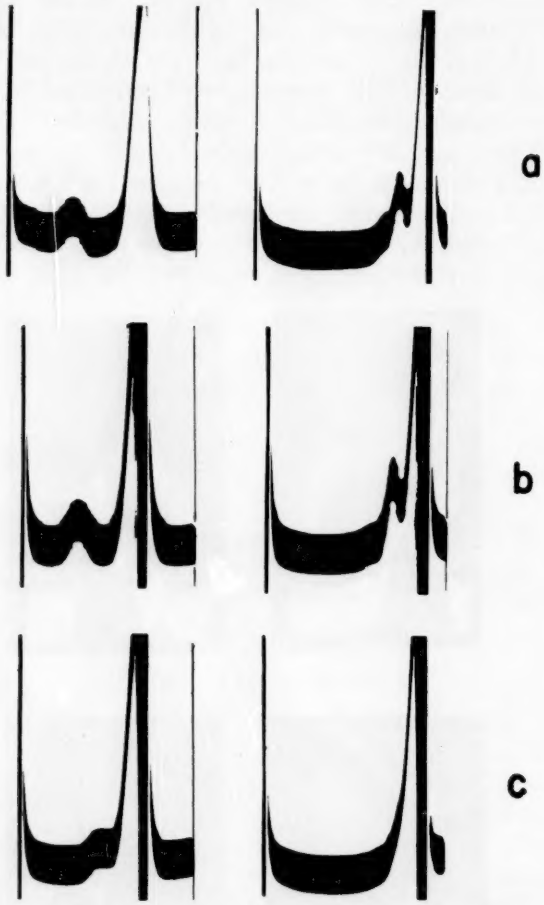

FIG. 2.—Ultracentrifuge patterns of (*a*) unacetylated and (*b*) acetylated BSA–anti-BSA complexes and (*c*) a mixture of separately acetylated BSA and rabbit γ-globulin, at total protein concentrations of (*a*) 24.5, (*b*) 31.8, and (*c*) 27.9 mg/ml. Sedimentation proceeds to the left. Two patterns from each experiment, the later one on the left, are shown.

which sediment more rapidly than does acetylated γ-globulin in a mixture with separately acetylated BSA[10] (Fig. 2, *c*). Therefore, Ab activity has been largely retained.

DISCUSSION

The following facts emerge from these experiments. On extensive guanidination and acetylation of BSA, most of its activity as a precipitating antigen is retained, whereas acetylation under the same conditions of anti-BSA antibody causes practically a total loss of its activity. That this loss of activity is due to

chemical reaction at the Ab reactive sites, and not to nonspecific effects, is shown by the protection[11] against acetylation conferred upon Ab by the presence of an excess of BSA. The presence of an excess of the nonspecific protein ovalbumin, on the other hand, does not protect anti-BSA.

Since acetylation under these conditions is highly specific for free amino groups[7] and since there are two reactive sites per Ab molecule,[8] and it is likely that there is only one positively charged group per site,[1] we infer that there are two amino groups per anti-BSA Ab molecule which are critically present in the reactive sites. If the studies of Porter[12] on the rabbit anti-ovalbumin system may be extrapolated to the anti-BSA system, it appears that the one N-terminal amino acid (alanine) per Ab molecule is not involved in the Ag-Ab bond. Hence it is probably the ϵ-NH_2 of a lysine residue that is present in each Ab site.

Pressman and Sternberger[4] first found that artificial homologous haptens specifically protected antihapten antibodies against inactivation by iodination. In our experiments, protection against chemical inactivation of antibodies by their homologous natural protein antigens has been demonstrated for the first time, and the much more specific acetylation reaction has been used. These protection experiments point the way to future studies of the structure of the reactive sites of Ab. In the presence of excess BSA, the available free amino groups of anti-BSA Ab may be exhaustively acetylated, except for those in the reactive sites specifically protected by the Ag. If the acetylated but still active Ab can then be purified away from the Ag, the active amino groups can be labeled with a reagent such as DNFB; isolation and characterization studies[13] of the DNP-amino acids and DNP-peptides might then be feasible. Such investigations are now under way in our laboratory.

* Chemistry Department Contribution No. 1342.

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[9] Acetylation not only markedly increases the negative electrophoretic mobilities of BSA and γ-globulin but also produces a splitting of the BSA boundary (Fig. 1, boundaries *1* and *2*). On acetylation of the complexes, some Ab is apparently inactivated, and a small peak migrating at the rate of acetylated γ-globulin (Fig. 1, *a*, boundary *5*) is observed which is not present in the unacetylated complexes. Also, in Fig. 1, *b*, the presence of a small peak roughly corresponding in mobility to boundaries *3* and *4* in Fig. 1, *a*, is due to the small amount of β-globulins left in the γ-globulin fraction upon $(NH_4)_2SO_4$ fractionation.

[10] Acetylation causes a reduction in sedimentation constants of BSA and γ-globulin. While the ultracentrifuge patterns of Fig. 2, *b* and *c*, were taken under identical conditions, those of Fig. 2, *a*, were chosen so that the free antigen boundary has sedimented the same distance as in Fig. 2, *b* and *c*.

[11] Since Ag and Ab are in equilibrium, the significance of the term "protection" is properly that the *rate* of acetylation of the Ab sites is markedly diminished by the presence of Ag.

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RADIATION DAMAGE IN THE DEVELOPING GERM CELLS OF DROSOPHILA VIRILIS

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Since 1945 a great many studies have been made to determine the relation between the amount of damage induced by ultraviolet or ionizing radiations and the chemistry of the cell. One important biological activity which involves both chemical and genetic changes in the cell is the process of maturation with gamete formation. Alexander[1] has presented new data and reviewed older experiments which prove that the mutation rate per roentgen unit X-ray dose is lower in spermatogonia than in mature sperm of *Drosophila melanogaster*. Auerbach[2] and Lüning[3] had shown that the mutation rate and rearrangement rate varied during the maturation cycle. Stone, Haas, Alexander, and Clayton[4] showed that major differences in rearrangement rate occurred through the maturation cycle of *Drosophila virilis* males. The frequency of rearrangement was influenced by the chemistry of the cell. This paper presents data showing the relation of genetic damage to dosage and to differences in gaseous environment.

Materials and Methods.—Strain No. 1801.1 of *D. virilis* from Texmelucan, Mexico, was used as the standard stock. The mutant marker stock used to score translocations contained *broken* (*b*, on chromosome 2), *tiny bristle, gapped* (*tb, gp*, on 3), *cardinal* (*cd*, on 4), and *peach* (*pe*, on 5). The microchromosome 6 and the X chromosome were not marked, but translocations involving the Y were checked. Chromosome damage was scored genetically from backcrosses as translocations produced between any two (T_2) or more (T_3, T_4, etc.) major chromosomes (Y, 2, 3, 4, and 5). Dominant lethals were determined from the number of eggs laid that failed to develop into pupae. In some crosses samples of freshly laid eggs were examined for sperm, using the smear technique devised by Patterson, Stone, and Griffen.[5] This differentiates between lack of development due to dominant lethals and that due to unfertilized eggs (see experiment 12).

All tests were carried out at 1–3° C. Pretreatment, irradiation, and posttreatment were done in the same gases, except in experiment 13*b*, where CO replaced O_2 in posttreatment (see Table 2). The dose to adults was delivered at 1,000 r per minute (250 kv., 15-ma. X-rays) for the necessary interval, except in experiment 15, where two 500-r doses were separated by one minute. The pupae received 1,000 r at 173 r per minute from an X-ray machine operating at 50 kv., 5 ma., with a 1-mm. aluminum filter. Tests using CO were carried out in the dark.

When adults were tested (experiments 12, 13*a*, 13*b*, 14, and 15), males 19–21 hours old were X-rayed and mated individually to three mature females. After the 5 days required for *D. virilis* males to mature, the males were remated individually to three mature females, consisting of one normal and two marker females. The males were remated to three such females every 2 days for eight periods. After the male was removed, the two types of females were separated. The number of eggs laid by the normal females were counted each day for 3 or 4 days and com-

pared with the number of pupae that developed as a measure of dominant lethals. F_1 males from the marker females, usually six to ten from each P_1 male, were backcrossed to marker females, and translocations were scored in F_2 from unusual genetic linkages. The fertility of F_1 males was recorded to be sure that the data were not biased by excessive sterility. The first period, A, represents the first effective sperm samples obtained from mature males, and the testing continued through period H, when the males were 20–21 days old. This procedure tests gametes irradiated at different stages of development from nearly mature sperm (A) back to spermatogonia (H).

In tests 17 and 18, old larvae were allowed to pupate on filter paper over a 20-hour interval and then were set aside for 90 hours. These 90–110-hour pupae were divided into two lots: one was pretreated 8 hours in N_2, irradiated with 1,000 r, and then posttreated for 2 hours; the other was treated similarly, except that CO was used in place of N_2 throughout the test.

Results.—The results of the tests are given in Tables 1 and 2. Most of the information is plotted on Figures 1–4. Experiment 12 is taken from an earlier paper[4] to allow a three-point dosage comparison. Three control egg-development tests

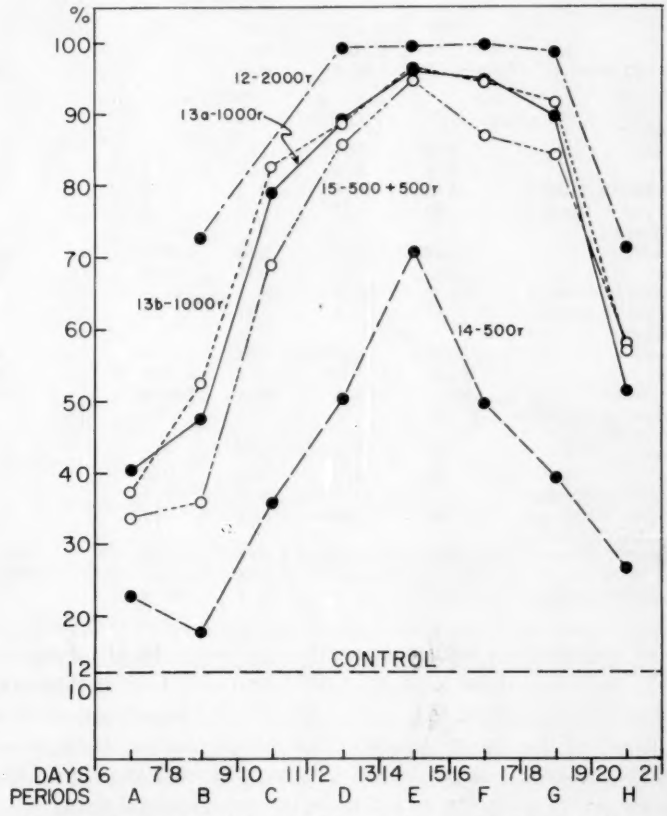

FIG. 1.—The relation between X-ray dosage (in O_2) and the frequency of dominant lethals produced in different stages of the developing male germ cell.

were run (Table 1). The first two were run like the experimental series. In these two, 12.0 per cent of 27,081 eggs failed to develop. This 12.0 per cent control level is indicated on Figure 1. Dominant lethals due to irradiation account for the major fraction of deaths in the experimental series. The third test was made with a newly selected, very viable stock to determine whether the egg development on successive days still showed the increase found in the first controls and in the experimental material. There was no obvious increase in egg development as the male aged, as shown by comparing the productivity of the first female mated with that of the second female mated the next day, etc. There was a consistent increase in hatch on successive laying days from all females.

TABLE 1

CONTROL EGGS DEVELOPMENT TESTS

	Laying Day					
	1st	2nd	3d	4th	5th	Total
			First Test			
Pupae	2,768	2,533	1,375	741	616	8,033
Eggs	3,506	2,885	1,467	787	636	9,281
Per cent to develop	79.0	87.8	93.7	94.2	96.9	86.55
			Second Test			
Pupae	7,271	5,840	2,685	...	...	15,796
Eggs	8,524	5,439	2,837	...	...	17,800
Per cent to develop	85.3	90.7	94.6	...	...	88.74
			Third Test			
First female:						
Pupae	1,407	581	147	410	...	2,545
Eggs	1,475	599	148	414	...	2,636
Per cent to develop	95.4	97.0	99.3	99.0	...	96.5
Eggs per ♀ per day	40	17	8	15	...	20
Second female:						
Pupae	1,303	374	528	405	...	2,610
Eggs	1,357	383	534	410	...	2,684
Per cent to develop	96.0	97.7	98.9	98.9	...	97.2
Eggs per ♀ per day	50	14	20	18	...	26
Third female:						
Pupae	533	493	428	199	...	1,653
Eggs	549	504	431	199	...	1,683
Per cent to develop	97.1	97.8	99.3	100.0	...	98.2
Eggs per ♀ per day	32	23	21	11	...	22
Fourth female:						
Pupae	746	419	238	215	...	1,618
Eggs	798	439	240	220	...	1,697
Per cent to develop	93.5	95.4	99.2	97.7	...	95.3
Eggs per ♀ day	40	22	13	13	...	22
Average, third test:						
Pupae	3,989	1,867	1,341	1,229	...	8,246
Eggs	4,179	1,925	1,353	1,243	...	8,700
Per cent to develop	95.5	97.0	99.1	98.9	...	96.9

The rate of spontaneous translocations is very low. In all of our tests so far published,[4, 6, 7] including these tests, we have found only four spontaneous translocations. These were detected as a cluster of identical translocations from a tested F_1. Three involved the chromosomes of the female marker stock, which was not X-rayed; in the other, all progeny of an F_1 male carried the same translocation. This spontaneous rate is too low to influence the experimental data.

The results of experiments 12–15 are in agreement, whether radiation damage is assayed as recoverable translocations or as dominant lethals (Table 2 and Figs. 1

and 2). The first sperm utilized (stage A, when the males are 6–7 days old) were nearly mature sperm at irradiation. Clayton (unpublished) reports that the tails of the sperm in *D. virilis* males less than 24 hours old have not coiled, and therefore some processes in sperm development are incomplete. Dominant lethals increased in frequency from stage A to stage E and then decreased until stage H (Fig. 1). Even at 500 r all stages show a frequency of lethals above control values.

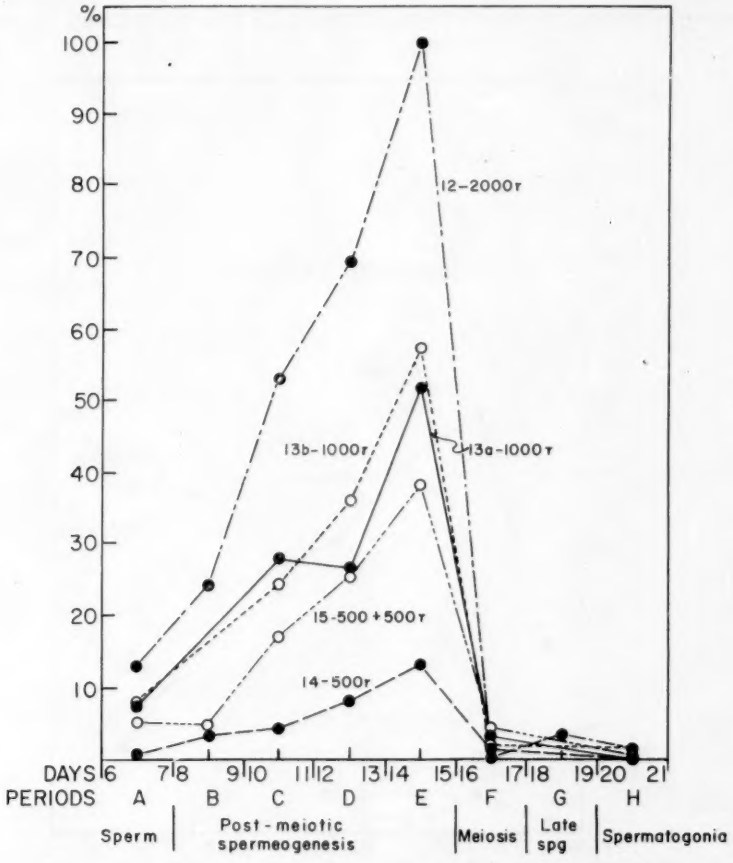

FIG. 2.—The X-ray dosage (in O_2) translocation frequency in the male-germ-cell cycle. The approximate limits of the different stages are shown.

The damage at 2,000 r was too great to show relations from stage C to stage G, as too few survived in these stages, but a direct relation to dosage still existed in stage H.

The translocation rate showed the same increase in damage from stage A to stage E, then dropped to around 1 per cent for stages F, G, and H. These last three stages showed that marked differences in the two measurements of genetic damage for dominant lethals were frequent when few translocations occurred. In both types of test the maximum damage occurred in period E, 14–15 days after the 19–21-hour-old males were irradiated.

The 4–5-day-old pupae in experiments 17 and 18 gave similar curves for dominant lethals and translocations, but the period of peak damage was shifted from stage E to stage B (Fig. 3). Damage measured as dominant lethals continued through period F, but the material treated in nitrogen (experiment 17) was about control level by period G. This is the only test where damage, measured as dominant lethals, drops to the control level. Damage was produced at all stages where the pupae were irradiated in carbon monoxide, even in period G (experiment 18). The absence of oxygen in these two experiments (17 and 18) reduced the radiation

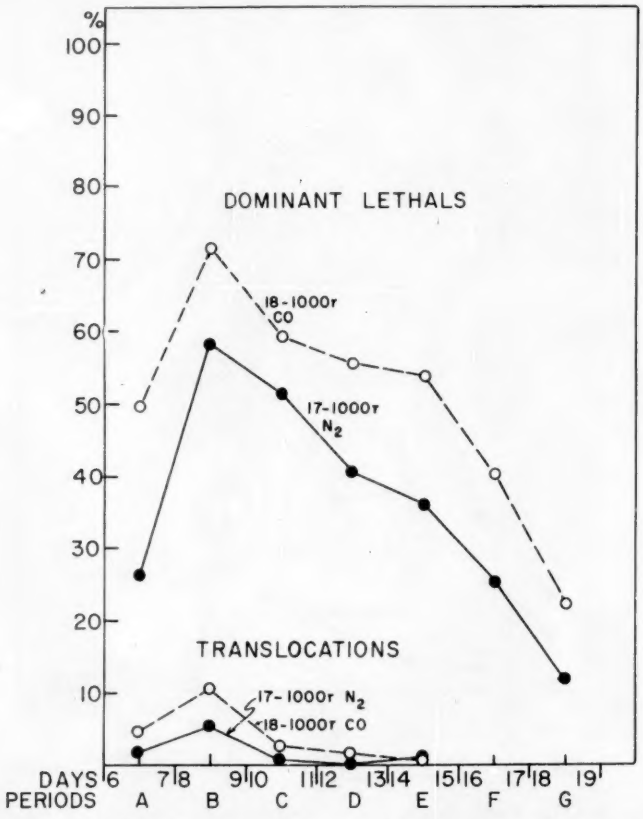

FIG. 3.—The genetic damage in the different stages from irradiating pupae (in N_2 or CO).

effect to a much lower level than in the other tests using 1,000 r. Carbon monoxide increased the damage above that in nitrogen at all stages during the meiotic cycle. Neither of these tests of treated pupae show the characteristic reduction in P_1 male fertility in periods F and G found in the adult 1,000-r test (experiments 13*a*, 13*b*, and 15).

Discussion.—The work on the meiotic cycle in *Drosophila* has been reviewed by Cooper.[8] The designation of the meiotic cycle in *D. virilis*, shown on Figure 2, is approximated by analogy with other insects, including *D. melanogaster*. The vari-

ation in response to irradiation is not restricted to insects but is also characteristic of plant material, as was shown in *Trillium erectum* for rearrangements by Sparrow[9] and in maize for mutations by Singleton.[10] Bonnier and Lüning[3, 11] demonstrated a cycle of sensitivity during spermatogenesis both for breaks and for mutations in *D. melanogaster*. Auerbach[2] established differences in mutation frequency in relation to stages of the meiotic cycle in *D. melanogaster*. She used crossing over in the male induced by the X-ray treatment to prove which meiotic stage gave a particular mutation frequency. Adult males were irradiated, placed individually

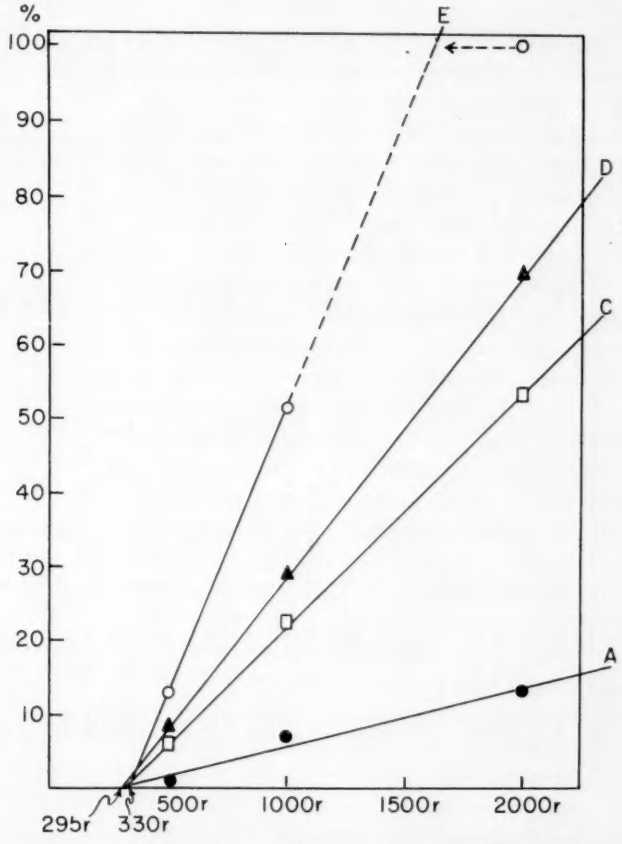

FIG. 4.—The direct proportionality of translocation frequency to X-ray dosage above a minimum in the susceptible postmeiotic stages. The lines are fitted by least squares.

with three virgin females, and transferred to three fresh females at 3-day intervals through four consecutive periods, *a*, *b*, *c*, and *d*. Auerbach gave results for both sex-linked and autosomal lethals and mentioned that gross rearrangements followed the same pattern as mutations. The peak mutation rate, both sex-linked and autosomal, occurred in period *b*, and the peak of sterility in period *c*. Single recombinants from crossing over were absent from period *b* but were present in period *c*, while complementary classes and bundles of identical crossovers were present only

TABLE 2

EFFECT OF DOSAGE AND GAS ENVIRONMENT ON RADIATION DAMAGE TO THE DEVELOPING GERM CELLS OF *D. virilis*

EXPERIMENT	PERIOD*	PER CENT P_1 MALES FERTILE	TYPES OF TRANSLOCATIONS						TOTAL SPERM TESTED	PER CENT SPERM WITH TRANSLOCATIONS ± 2 S.D.	DOMINANT LETHALS			PER CENT EGGS FERTILIZED
			T_2	T_3	T_{2+2}	T_4	T_{2+3}	T_5			Eggs	Pupae	Per Cent Lethals	
13*a* (1,000 *r*, O_2; post-treatment, O_2)	A	86	21	3	1	...	...	...	342	7.3± 2.5	3,758	2,233	40.6	...
	B	100	...	...	...	...	...	...	...	.....	5,972	3,133	47.5	...
	C	100	49	13	2	3	...	...	240	27.9± 5.9	3,892	818	79.0	...
	D	99	38	12	6	2	...	...	218	26.6± 6.0	5,399	574	89.4	...
	E	89	19	15	2	5	...	...	79	51.9±11.9	4,781	189	96.0	...
	F	67	1	1	...	...	...	...	63	3.2± 2.4	3,171	163	94.9	...
	G	68	...	...	...	...	...	...	...	.....	2,678	274	89.8	...
	H	89	...	...	...	...	...	...	143	0.0	2,068	1,009	51.2	...
13*b* (1,000 r, O_2; post-treatment, CO)	A	80	24	1	...	...	...	...	306	8.2± 3.0	2,966	1,866	37.1	...
	B	93	...	...	...	...	...	...	...	.....	5,545	2,617	52.8	...
	C	97	48	7	1	3	...	1	245	24.5± 5.5	4,244	744	82.5	...
	D	97	58	14	1	4	2	1	222	36.0± 6.5	5,411	599	88.9	...
	E	95	36	16	3	6	...	...	106	57.5± 9.7	5,690	203	96.4	...
	F	62	1	...	...	...	...	...	56	1.8± 3.6	2,405	124	94.8	...
	G	65	...	...	...	...	...	...	...	.....	2,236	185	91.7	...
	H	80	1	1	...	...	...	...	140	1.4± 2.6	2,397	1,036	56.8	...
15 (500 r + 500 r, O_2)	A	79	15	...	...	...	...	...	294	5.1± 2.6	3,835	2,542	33.7	...
	B	87	14	...	...	...	...	...	286	4.9± 2.6	5,513	3,536	35.9	...
	C	91	39	5	2	...	...	...	266	17.3± 4.7	5,480	1,651	69.1	...
	D	92	55	11	...	3	...	...	273	25.3± 5.2	6,386	914	85.7	...
	E	81	9	6	2	1	...	...	47	38.2±14.6	4,334	228	94.7	...
	F	83	1	1	1	...	...	...	69	4.3± 4.9	2,018	262	87.0	...
	G	67	...	...	...	...	...	...	...	.....	2,823	446	84.2	...
	H	85	1	...	...	...	...	...	112	0.9± 1.8	3,638	1,543	57.6	...
14 (500 r, O_2)	A	94	2	...	...	...	...	...	303	0.7± 1.0	4,585	3,533	22.9	...
	B	91	10	...	...	...	...	...	297	3.4± 2.0	4,996	4,115	17.6	...
	C	96	11	...	...	...	...	...	253	4.3± 2.6	5,463	3,513	35.7	...
	D	94	25	2	...	...	...	...	333	8.1± 3.0	6,047	2,999	50.4	...
	E	93	24	6	1	...	...	...	236	13.1± 4.4	4,890	1,424	70.9	...
	F	93	4	...	...	...	...	...	263	1.5± 1.6	3,181	1,602	49.6	...
	G	90	...	...	...	...	...	...	...	.....	3,544	2,153	39.2	...
	H	91	...	...	...	...	...	...	111	0.0	2,633	1,927	26.8	...
12‡ (2,000 r, O_2)	A	73	62	6	2	...	...	...	525	13.3± 3.0	...	...	...	...
	B	89	111	17	7	4	...	...	574	24.2± 3.6	15,198	4,106	73.0	91.9
	C	33	14	8	1	1	1	...	47	53.2±14.5	...	...	...	...
	D	29	2	2	1	3	...	1	13	69.2±25.6	19,731	147	99.3	95.3
	E	26	4	1	1	1	...	1	8	100.0	21,456	127	99.4	96.8
	F	7	...	...	...	...	...	...	1	0.0	9,688	22	99.8	93.9
	G	8	1	...	...	...	...	...	27	3.7± 7.2	8,479	117	98.6	36.4
	H	69	2	...	...	...	...	...	150	1.3± 1.8	9,111	2,615	71.3	81.3

	A	97	4	...	...	247	1.6± 1.6	5,674	4,186	26.2
	B	97	11	1	...	218	5.5± 3.1	4,513	1,877	58.4
17 (1,000 r [173 r per minute], N_2)	C	97	1	...	...	116	0.9± 1.7	6,400	3,128	51.1
	D	95	...	...	...	101	0.0	5,578	3,315	40.6
	E	97	1	...	...	98	1.0± 2.0	4,681	2,989	36.1
	F	98	...	...	...	...	...	5,034	3,763	25.2
	G	...	...	...	...	...	...	3,949	3,479	11.9
	A	76	12	...	...	267	4.5± 2.6	3,142	1,582	49.6
	B	91	22	1	...	212	10.9± 4.2	5,000	1,421	71.6
18 (1,000 r [173 r per minute], CO)	C	99	5	...	...	180	2.8± 2.4	6,532	2,670	59.1
	D	94	1	...	...	62	1.6± 3.0	4,650	2,080	55.3
	E	96	1	...	...	126	0.8±1.6	2,902	1,339	53.9
	F	91	...	...	...	...	...	4,979	2,961	40.5
	G	...	...	...	...	...	...	3,361	2,611	22.3

* Age of males in days during mating: A = 6–7, B = 8–9, C = 10–11, D = 12–13, E = 14–15, F = 16–17, G = 18–19, H = 20–21.
† From Stone, Haas, Alexander, and Clayton, *Univ. Texas Publ.*, No. 5422, p. 244, 1954.

in period *d*. Friesen[12] proved that X-ray-induced crossing over could occur at meiosis or in spermatogonia with bundles of identical classes from early spermatogonia. Auerbach concludes that the greatest frequency of mutation occurs in some stage of spermeogenesis after meiosis and that the peak sterility occurs in late spermatogonia and the early meiotic stage.

Sobels confirmed this cycle for *D. melanogaster*.[13] In addition, he found that azide and cyanide increased mutation rate particularly in the sensitive period. This sensitivity cycle is similar to that worked out by Bonnier and Lüning, except for differences due probably to differences in technique in certain experiments. Clark[14] demonstrated the cycle in *D. melanogaster* for sex-linked lethals and translocations. He showed with his experiments that variation in procedure modifies the distribution of abnormalities in successive broods. Auerbach's use of induced crossing over in the male established the relation between the meiotic stage and the amount of damage.

By analogy and by comparison with this other work, especially Auerbach's, with due regard for the slower tempo of development in *D. virilis*, we can approximate the stages in germ-cell development in these young males at irradiation. As indicated on Figure 1, stage A is nearly mature sperm; stages B, C, D, and E are postmeiotic spermeogenesis; stage F includes meiosis; stage G is late spermatogonia; and stage H is spermatogonia. If we compare stages H through F with the data for *D. melanogaster* females (Glass[15]) or *Sciara* females (Bozeman and Metz[16]) or *Habrobracon* females (Whiting[17, 18]), we find no essential difference. Where analyses are possible in these cases, there are a few translocations and a moderate number of inversions which survive and an increasing frequency of rearrangements leading to dominant lethals from spermatogonia to meiosis or from oögonia to the first meiotic meta- or anaphase. If stages homologous to stages A through E of the male occur in females, and the specialized processes in the development would seem to make sperm a special case, they have not been tested for irradiation effects.

Oakberg[19] showed that in mice late spermatogonia (type B) were very sensitive and therefore were destroyed by low doses of irradiation (LD_{50} *ca.* 25 r). Early sperma-

togonia (type A) are much more resistant, so that regeneration will occur after much higher doses. Spermatids and sperms are also more resistant. Pontecorvo found a similar situation in *Drosophila*.[20] The agreement between the mouse and *Drosophila* explains stage G (experiment 12), which we regard as late spermatogonia. There were so few sperm available to the inseminated females that only part of the eggs were fertilized.

In young males the number of dominant lethals rises rapidly from early spermatogonia through meiosis (stages H through F) and into spermeogenesis to the stage following meiosis (stage E); then it falls again. Translocations are very infrequent until after meiosis (stage F) and then rise rapidly to a peak in stage E. Not all reasons for the difference are clear, but Deschner and Sparrow[21] have found the same type of situation on irradiating *T. erectum* either with X-rays or with thermal neutrons. The amount of breakage (measured as fragments) and the amount of rejoining (measured as dicentric and ring chromosomes) varied independently. Similar differences in *Drosophila* in these independent variables will explain the presence of few translocations despite many dominant lethals through meiosis. Probably conditions are much more favorable for rejoining in the normal gene order or joining as a rearrangement in the postmeiotic stages of sperm formation. Deschner and Sparrow discuss some of the factors which might contribute to these differences.

There is another factor which may be important. The sperm of animals undergo a unique change in chromosome composition, for the usual histone protein is replaced by protamine protein.[22] The high susceptibility of stage E to irradiation damage may be due to the fact that this is the most active stage of protein reorganization as well as of cytoplasmic changes, and the chromosome is more subject to breakage in these unprotected and changing conditions.

Most of the tests in Table 2 were run under similar conditions, using different doses of radiation to test the consistency of the cell cycle and the dose response. Figures 1 and 2 show that the cycle was consistent when measured as dominant lethals or recovered translocations. The frequency of dominant lethals was too great at higher doses to assess the relation to dose. The translocation tests of stages A, C, D, and E from Table 1 and Figure 2 were used to illustrate the relation between X-ray dosage and translocation frequency (Fig. 4). Stages F, G, and H (meiosis back to spermatogonia) were not used, for there were too few translocations. The lines in Figure 4 are fitted to the points by least squares. Only stage A, which is most nearly equivalent to mature sperm in an aged male, might be fitted better by an S-shaped curve. All these curves which best fit these points intersect the base line between 295 and 330 r. The 2,000-r value of 100 per cent translocations for stage E must be disregarded, for there were no intermediate doses between 1,000 and 2,000 r. Many or most of the translocations must result from two or more hits even at the most susceptible stages in spermeogenesis. After the minimum necessary amount of radiation in these susceptible stages, the number of translocations seems to increase proportionally with dosage. The damage measured as translocations to these stages of spermeogenesis with X-rays resembles that of neutrons to mature sperm, except that the damage from the latter is directly proportional to dosage down to zero.[7]

Patterson, Brewster, and Winchester showed that mature eggs of *D. melanogaster* were more susceptible than immature eggs when radiation damage was

measured as dominant lethals.[23] This makes *D. melanogaster* females similar to *Sciara* and *Habrobracon*. Glass[15] found that there was a much higher frequency of inversions than of translocations produced by irradiating *D. melanogaster* females, showing that the material responded much like *Sciara*. He found an increase in translocations and Minutes present in irradiated males from the third brood (8–11 days after irradiation) similar to that found by Bonnier and Lüning and by Auerbach for male germ cells. Glass found that the number of Minutes was much smaller in the third brood from irradiated females, again showing greater resistance of earlier stages in oögenesis in *Drosophila*, again like *Sciara* and *Habrobracon*. It is obvious that mature spermatozoa differ from unfertilized *Drosophila* eggs, but the comparison is not between equivalent stages. Alexander showed that spermatogonia had a much lower mutation rate than mature sperm.[1] In order to compare males and females, comparable stages in gametogenesis should be used. Mature eggs prior to fertilization stop at metaphase or anaphase of the first maturation division. If we compare the results in females with those in males for stages H through F, they are very much alike.

In an earlier test, 2–3-day pupae were irradiated with 2,000 r in 95 per cent CO + 5 per cent O_2.[4] Wolsky[24] studied the inhibition of oxygen consumption of the pupae caused by CO and found that higher concentrations of CO were more effective at all stages of pupal development. He showed that CO inhibition of respiration in *Drosophila* pupae was light-reversible. Wolsky suggested that changes in amount or activity of the Warburg-Keilin system might explain the change in respiration during pupation. Bodenstein and Sacktor showed that the cytochrome c oxidase activity is reduced drastically in early pupation.[25] The low point of this U-shaped cycle is about 2–3 days after the onset of pupation; then cytochrome c increases to the normal adult amount shortly after eclosion of the adult. In our earlier irradiation tests no offspring were produced in stage A, and damage measured as translocations was great in stages B and C and then fell to 0 in stages D, E, and F.[4] The Texmelucan *D. virilis* strain takes 7 days in the pupal stage when irradiated, and the 4–5-day-old pupae used in the present experiment should have about half the amount of cytochrome c found in the young adult. Pupae were pretreated in N_2 (experiment 17) or in CO (experiment 18) for 8 hours prior to irradiation, so that there was very little residual O_2 present during irradiation. Nevertheless, the damage was consistently higher in CO than in N_2. Therefore, the cytochrome system or other agents inhibited by CO protects the chromosomes against damage which does not depend on an appreciable concentration of O_2. This suggests Barron's conclusion that OH is reacting with cytochrome.[26] King, Schneiderman, and Sax reported that both CO and CO_2 increased radiation damage when present with O_2.[27] Similar results were obtained with *Drosophila* by Haas, Dudgeon, Clayton, and Stone.[6] However, Schneiderman and King showed that CO_2 alone was equivalent to radiation in a vacuum. Therefore, the increase in damage occurred when CO_2 acted synergistically with O_2. In tests 17 and 18 with pupae, the CO inhibits the cytochrome c system or some other active enzyme system which normally protects the chromosome against radiation damage. Even in the absence of O_2 from external sources, the inhibition of the cytochrome c system by CO increased the radiation damage measured as dominant lethals or translocations above that when the material was treated in N_2

(Fig. 3). Despite this effect of CO, damage was much less than in other 1,000-r tests.

The cycle was changed so that the peak fell in stage B. Even allowing for the fact that the pupae were irradiated 3–4 days earlier than the young adults, the peak still fell one period earlier than expected. We presume, by analogy with Auerbach's results, that this is the result of difference in amounts of mature sperm which were disposed of before earlier stages could move through.

The pupae in these tests (17 and 18) were irradiated at a lower intensity (173 r per minute) than the adults (1,000 r per minute). Sax, Luippold, and King presented tests that showed an effect of both intensity and fractionation on rearrangement rate in *Tradescantia*.[28] Haas *et al.*[6] demonstrated that there was also an intensity effect in *Drosophila*. When experiment 15 is compared with experiments 13*a* and 13*b*, it suggests a fractionation effect in stages of spermeogenesis of *Drosophila*, but more tests are necessary to prove this point.

A volume on mutation induction in plants, carried out by the group in Sweden led by Gustafsson, has recently appeared. Ehrenberg and Nybom discussed the direct and indirect effects of radiations of different sorts in that volume.[29] There are many parallels between their results with plants and our work on *D. virilis*.[4, 6, 7, 30] The current tests show that irradiating with poisoning of the cytochrome system with CO in the absence of an external source of oxygen causes more damage than irradiating in N_2 (experiments 17 and 18). Furthermore, replacement of O_2 by CO immediately after irradiation for a 30-minute period causes a little change in the amount of damage during the cycle, for the results from experiments 13*a* and 13*b* are very similar.

Whiting[18] has recently reported on new experiments on the effect of oxygen on irradiation effects in *Habrobracon*. She concludes that oxygen increases breakage of chromosomes. Our evidence from *Drosophila* both for oxygen and for other factors which modify oxidative radicals is in agreement with that conclusion.

Summary.—The relationship between X-ray dosage and genetic damage was determined throughout the meiotic cycle of *D. virilis*. Damage was measured as dominant lethals and translocations. The number of aberrations varies from 20 to 100 fold between stages, depending on the physiological conditions at irradiation (Figs. 1–3). The period (E) just after meiosis is the most susceptible of the stages. The early stages of the maturation of the sperm (E, D, and C) are the periods of differentiation of this unique cell type. Along with the complex changes in the cytoplasm during this period, there occurs the replacement of the histone protein by protamine protein in the chromosomes. The susceptibility of these stages is markedly influenced by modifications in the environment, such as increased amount of O_2 or the presence of CO during irradiation.

During the susceptible period especially (stages A, C, D, and E, Fig. 4), the amount of X-ray damage measured as translocations is directly proportional to dosage above 300 r. Very few translocations are produced in premeiotic stages, although many dominant lethals occur. The late spermatogonia (stage G) are so susceptible to radiation injury that many cells in this stage die and few sperm are produced. Radiation damage to the different stages of oögenesis in the female *Drosophila* has been shown to be similar to that in *Sciara* and *Habrobracon*. The comparable stages in the male, stages H through F, respond to radiation very much

as the female. Stages equivalent to stages E through A and mature sperm are not present in the female. The enzyme activity as modified by the gaseous environment influenced the amount of damage to pupae (Fig. 4). Even without an external source of O_2, irradiation in CO modified the internal environment and increased radiation damage above that in N_2 at all stages. The sensitive stages of the maturation cycle in the male *Drosophila* are particularly useful in studying the relation between physiological activity and genetic damage from radiations.

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*A POSSIBLE MECHANISM FOR THE REPLICATION OF THE HELICAL STRUCTURE OF DESOXYRIBONUCLEIC ACID**

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Desoxyribonucleoprotein, the genetically active complex of the cell, carries on two important functions: that of self-replication and that of maintaining a cellular environment compatible with its own existence. An adjunct to the latter function among the differentiated cells of the multicellular organism is the accommodation of these processes within individual cells to the needs of the total organism. At present very little is known, at the molecular level, of the manner in which these processes take place. Recent advances in the knowledge of the chemical structure of this complex, however, make it possible to formulate a scheme for the replication of DNA which accords with the present-day conceptions of the complex and its cytological behavior during duplication of the chromosomal material prior to cell division.

On the basis of X-ray diffraction studies and the chemical composition of the complex, the desoxyribonucleoprotamine molecule has been pictured by Feughelman *et al.*[1] as a triple coaxial, relationally coiled helix. Two relationally coiled polynucleotide strands (*o* and *o'* in Fig. 1, *a* and *b*) are connected across the center of the helix by hydrogen bonding through the bases, guanine being linked to cytosine and adenine to thymine as proposed by Watson and Crick.[2] A third chain, a polypeptide, is relationally coiled about the two polynucleotide strands in such a manner that the ϵ basic groups of adjacent amino acids, projecting in opposite directions but parallel to the axis of the helix, can form ionic linkages alternately with the phosphate groups of both polynucleotide helices. The two polynucleotide helices are therefore connected one with the other, both by hydrogen bonding through their complementary bases and by ionic linkage through the associated protamine (Fig. 1, *a* and *b*). The desoxyribonucleohistone complex is assumed here to be similar to the protamine counterpart. The differences between the protamines and histones are probably of degree rather than of kind. Histones are of an extremely variegated nature; their properties—molecular weight,[3] amino acid composition,[4] even their occurrence in tissues[5]—appear to overlap somewhat with the less complex protamines. Both of these basic proteins form salt-like complexes with DNA. The protamine might be regarded as an extreme form of histone. The apparently globular nature of histone[6] can be compared with the more extended protamine configuration by assuming the globularity of the former to be due to an abundance of nonbasic loops such as were postulated by Feughelman *et al.*[1] to occur to a limited extent even in the otherwise extended protamine.

Replication of the polynucleotide in the desoxyribonucleoprotein complex can be visualized by first postulating a slight rotation or vertical displacement of one of the helices relative to the other, the structure more closely approximating that originally proposed by Watson and Crick,[2] in order to be able to accommodate additional strands of polynucleotide. Such a configuration would not contradict the results

of X-ray diffraction studies on the structure of this molecule,[1, 7] since, to date, these have been carried out on material obtained from nonproliferating tissues or from cells in which DNA doubling probably does not occur to any great extent. Figure 1, *a* and *b*, schematically illustrates the desoxyribonucleoprotein model as it

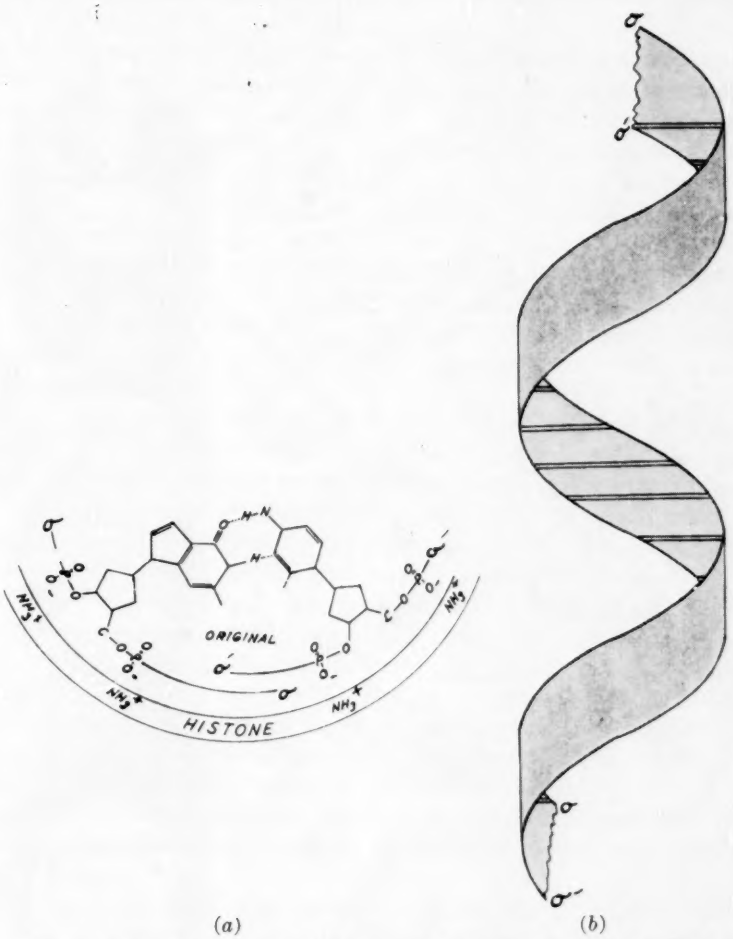

FIG. 1.—(*a*) Cross-section of the helical model of desoxyribonucleoprotein before replication, indicating the linking of the two polynucleotide helices (*o* and *o'*) by hydrogen bonding through their bases and by ionic linkage through the associated histone. The sugar was drawn coplanar with the bases here for ease of visualization. (*b*) Side view of the helical structure. The curved lines represent the polynucleotide strands; the horizontal lines, the hydrogen bonds; the shaded portion, the protein.

might appear at the onset of DNA duplication. Figure 1, *a*, shows the helix in cross-section. Throughout the following description this complex, consisting of polynucleotide helices *o* and *o'* and the attached histone, will be referred to as the "original." The newly synthesized polynucleotides *r* and *r'*, and histone, will be called the "replicate."

If, prior to replication, the hydrogen bonds are broken and a temporary distortion of the molecule enables the bases to rotate approximately 180° about the single nonresonating glycoside bonds, as indicated by the arrows in Figure 2, *a*, the bases would now be in position to combine by hydrogen bonding to complementary unpolymerized nucleotides, such as were demonstrated by Schmitz *et al.*[8] to be present

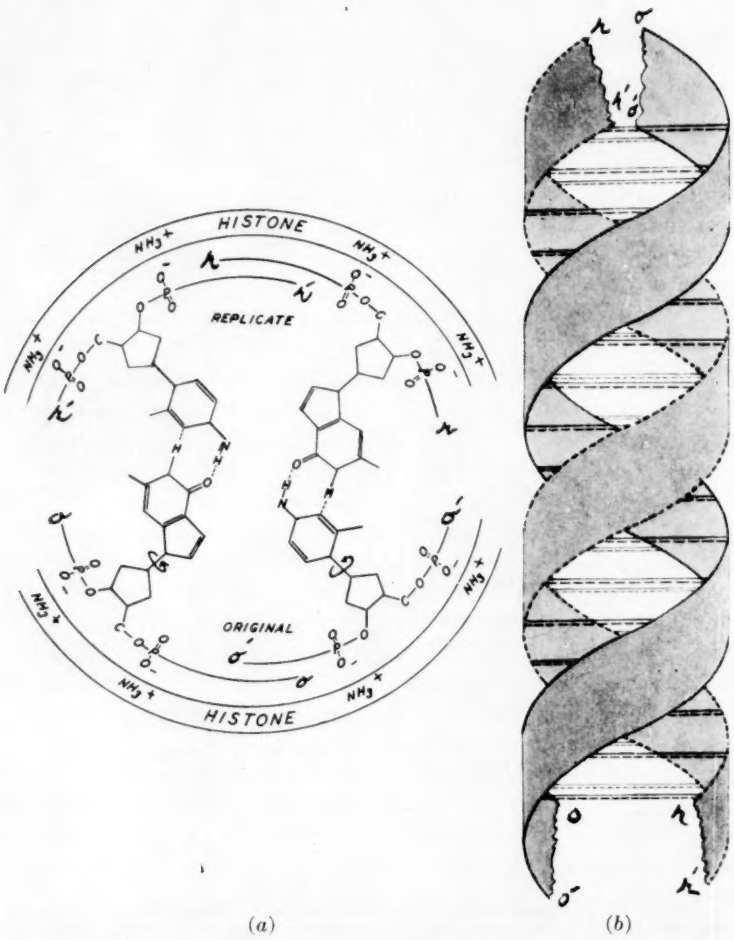

FIG. 2.—The helical model of desoxyribonucleoprotein during replication, showing the new position of the bases after rotation about the glycoside bonds and the relationships between the original and replicate components of the complex. The broken lines in *b* indicate the replicate polynucleotide helices.

in proliferating tissues. The original polynucleotides might meanwhile be held rigidly in place by means of their attachment through the histone (Fig. 2). In this manner the small molecules would be oriented for synthesis of new polynucleotide. The unpolymerized nucleotides, once aligned, would be expected to have a rotational configuration of the base about the glycoside bond similar to that of their

homologues in the original molecule, so that, after alignment, synthesis of the nucleotide bond would result in two new replicate polynucleotide helices. The *o* strand of the original would now be associated with the *r'* strand of the replicate, and *o'* of the original with *r* of the replicate. What had been the "left-hand" helix in the original association is now the "right-hand" helix in the new association between the original and the replicate, and vice versa.

Bloch and Godman[9] and Alfert[10] have shown that during the interphase period, while duplication of chromosomal material takes place, there is a simultaneous and parallel synthesis of both DNA and histone. This may reflect a simultaneous or rapid succession of the syntheses of both substances at the same site on the molecule. The complex, after duplication of chromosomal material but before separation, might then consist of four relationally coiled polynucleotide strands and two relationally coiled polypeptide strands. Each of the original polynucleotide strands would be associated with its complementary replicate strand by hydrogen bonding through the bases. The original strands remain in association with each other by means of their attachment to the original basic polypeptide; the replicate polynucleotides become similarly associated by means of attachment to the newly formed polypeptide (Fig. 2).

At a later stage, the hydrogen bonds between the original and replicate DNA might again be broken, and, either prior to or following separation of the complexes, the bases could rotate about the glycoside bonds, assuming the proper orientation for hydrogen-bond formation within the respective complexes (Fig. 3, *a* and *b*).

Separation of the original from the replicate complex might be visualized by assuming the histone and polynucleotides to form a permanent association during the whole process of replication and separation, the hydrogen bonds maintaining the only temporary linkage. As suggested by Watson and Crick[11] and Gamow,[12] separation of the relationally coiled strands (in the present instance, relationally coiled complexes) may be made possible by the chromosome coiling which takes place during prophase prior to cell division.[13] Winding of a double relationally coiled helix about an axis perpendicular to the axis of the helix, once for every turn in the helix, allows separation[12] (Fig. 4). Of course, any such process leading to separation of originals and replicates would be greatly complicated where chromosomes consist of great numbers of such paired complexes, as may be the case among the higher organisms.

The scheme for replication of the desoxyribonucleoprotein complex as presented here can claim no direct evidence in its support. Partial evidence might be provided by X-ray diffraction studies on material from cell populations containing doubled amounts of DNA. A number of phenomena occur in the duplicating cell which can be explained in terms of such a scheme. Furthermore, this scheme can overcome a few of the objectional features of previously suggested mechanisms, based upon replication of separated single units[11] rather than of associated double units. If DNA replication follows separation of the original double polynucleotide helices, the single polypeptide strand would have a "choice" to make as to which polynucleotide strand to follow, unless the cell uneconomically discards the old histone fraction and synthesizes anew a double complement. Either of these alternatives is more complicated than the presently proposed mechanism. More important, a permanent association between the histone and polynucleotides might serve

to stabilize the helical structure after disruption of the hydrogen bonds during DNA replication.

The present scheme also accords with recent work as reported in preliminary form[14] which suggests that during separation of daughter-chromosomes the newly synthesized DNA goes to one chromosome and the original to the other. If, on the

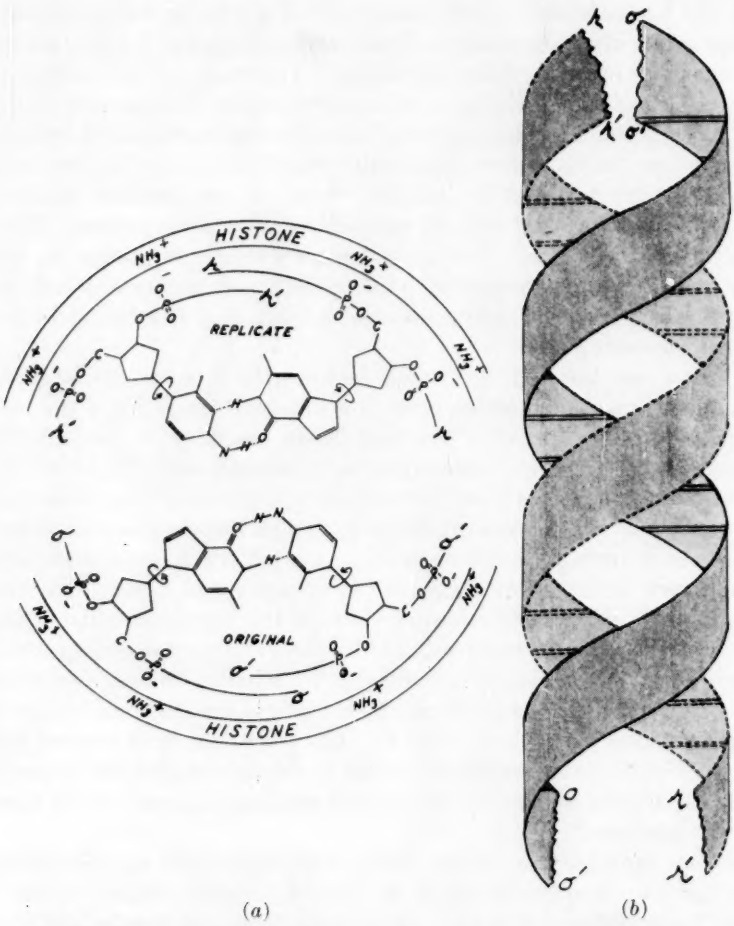

FIG. 3.—The helical model of desoxyribonucleoprotein after replication is complete, showing the bases in their original positions.

other hand, replication follows separation, the daughter-chromosomes would be expected to contain one strand of original for every strand of replicate material. The complete report of Plaut on the partition of old and new DNA during division will be of great interest.

If, as suggested above, chromosome coiling and contraction during mitosis are visible expressions of a separation occurring at a molecular level, the fact that DNA synthesis occurs during the interphase period[15] prior to visible signs of chromosome

coiling, rather than in the reverse order, would appear to support the present scheme of replication of DNA before separation of the helices.

Finally, histone has been proposed as a regulator of genetic expression among the

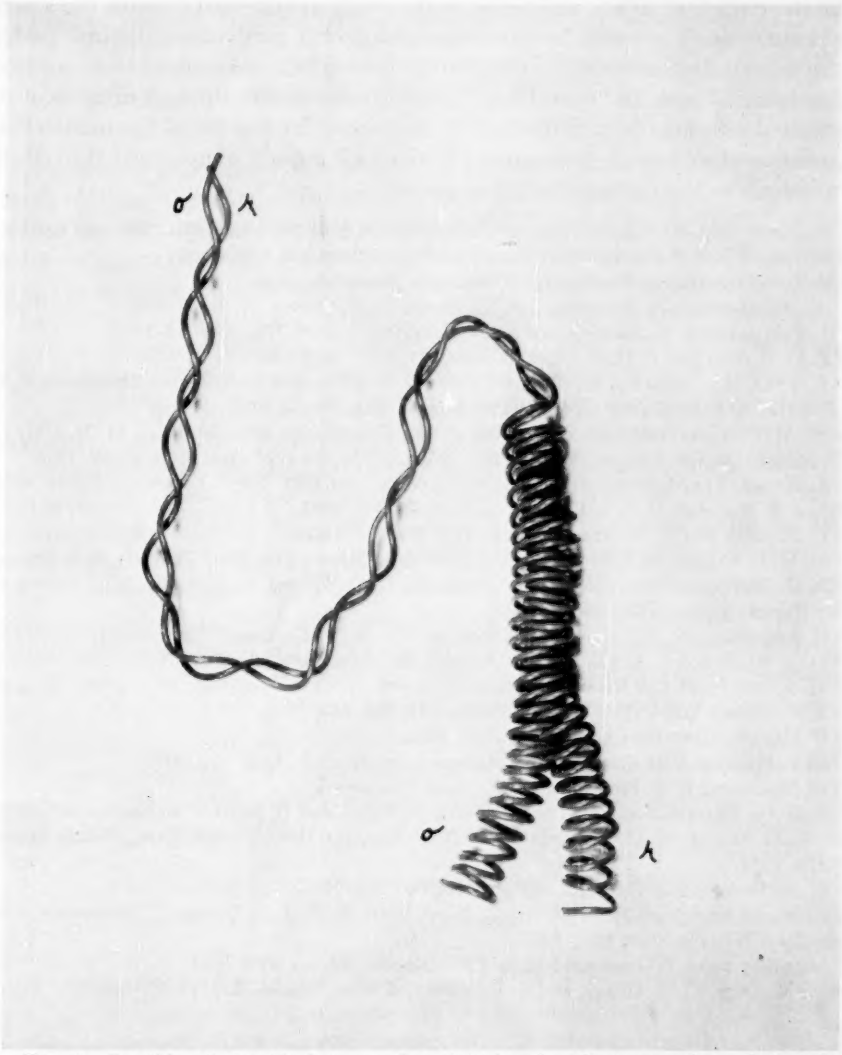

FIG. 4.—Possible relationship between the original and replicate complexes during the late interphase stage after duplication but before separation (*upper left*) and the mitotic stages as separation takes place (*lower right*), demonstrating how chromosome coiling could effect the separation of the two strands.

differentiated cells of an organism[16,17] the desoxyribonucleohistone complex being characteristic of a cell type much as DNA is characteristic of a species. This hypothesis is supported by evidence of a relative degree of histone constancy within cells of the same type[9,18] and, at the same time, histone variation between tissues,[16,18]

and low histone turnover rates,[19] *inter alia*. A permanent association of DNA with the histone during replication of DNA might provide the basis for replication of the histone also. If the nature of the histone attached to DNA at any one point could impress changes in the configuration of the DNA which could then be passed on to the replicated DNA, and hence to the newly synthesized histone, the scheme might conceivably provide for the transmission of a particular structural pattern of the desoxyribonucleohistone complex governing cellular activity. An accurate transmission of such an "operational" genetic complement through division of differentiated cells may be as important to the proper functioning of the multicellular organism as the accurate transmission of the total genetic complement through the germ cells is to the maintenance of a species.

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† Fellow of the Damon Runyon Fund for Cancer Research.

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SUPPRESSIVENESS: A NEW FACTOR IN THE GENETIC DETERMINISM OF THE SYNTHESIS OF RESPIRATORY ENZYMES IN YEAST

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In a series of papers from this laboratory[1] published since 1949, it was shown that clones of baker's yeast (*Saccharomyces cerevisiae*), whether diploid or haploid, constantly give rise during their growth to mutants ("vegetative 'petites' ") which are stable in vegetative reproduction and are characterized by a reduced colony size on media in which sugar is the limiting factor. The reduced colony size was shown to be due to a respiratory deficiency of the mutant cells,[2] due in turn to the lack of several enzymes (including cytochrome oxidase) bound, in normal yeast, to particles sedimentable by centrifugation.[3] It was suggested that, from the genetical point of view, the mutation resulting in the production of vegetative mutants consists in the loss or irreversible functional inactivation of a particulate cytoplasmic autoreproducing factor, required for the synthesis of respiratory enzymes.[4] This interpretation was based on the results of crosses which will be recalled below. It will suffice to say at this point that a single strain of vegetative mutants was utilized in the earlier detailed genetic study;[5] thus, until more recently, no systematic attempts had been made to compare the genetic behavior of a number of such mutants of independent origin. It is such a study, undertaken three years ago, that has now revealed that there exist at least two types of vegetative respiratory mutants which are, so far as we know, identical biochemically but which can be distinguished by their behavior in crosses with normal yeast. The purpose of this paper is to present a brief summary of the evidence for the existence of these two types of vegetative mutants, which, for reasons to appear below, will be referred to, respectively, as "neutral 'petites' " and "suppressive 'petites' " (the one previously studied[5] belonging to the former class).[6]

EXPERIMENTAL PROCEDURE AND SUMMARY OF RESULTS

A model experiment revealing the occurrence of two genetically different classes of vegetative "petites" and showing their differential characteristics is schematically represented in Figure 1, which will be referred to in the ensuing description.

A haploid strain, normal with respect to respiration (*N*) and carrying the recessive mutant genes *tr* and *his*, causing, respectively, tryptophan and histidine requirements, is plated out on complete medium (C.M.) in a Petri dish. Among the colonies produced, two small colonies are selected and subcultured; tests are performed on the resulting clones to ascertain that they represent stable respiration-deficient mutants. It will now be assumed that the two selected mutants happen to belong to the two extreme classes of vegetative mutants, i.e., that one of them is a neutral "petite" (*n*) and the other highly suppressive (*s*). We shall now examine the results of the crosses of these two mutants with a single strain of normal yeast of opposite mating type (the mating types of the strains involved are indi-

cated by the symbols a and α) carrying the recessive genes *met* and *ur* (methionine and uracil requirements).

The crosses are performed by the mass-mating technique,[7] i.e., by mixing a few drops of two cultures in a small volume of fresh complete medium contained in a test tube.

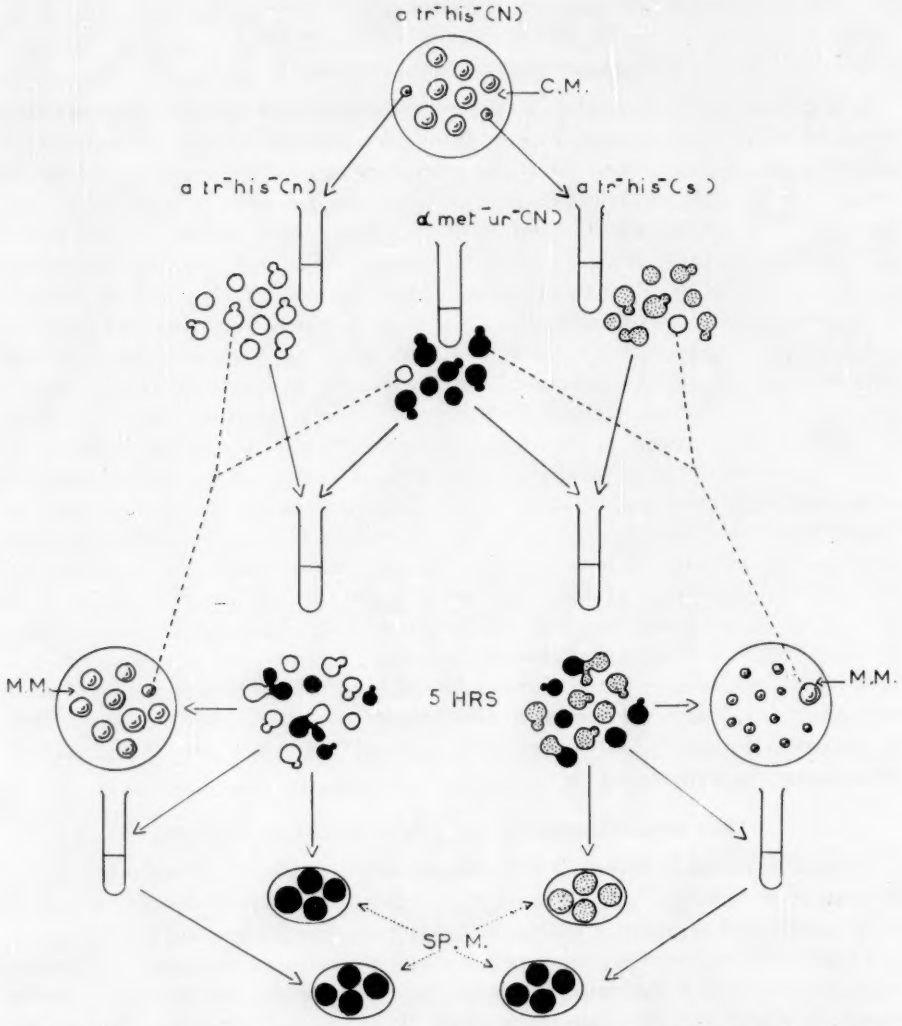

FIG. 1.—Design of a model experiment for the demonstration of the behavior of neutral and suppressive "petites" in crosses with a normal yeast. (*Black:* normal yeast cells; *strippled:* suppressive "petites"; *white:* neutral "petites.") Further explanations in text.

Five hours after the crosses are made, the mass-mating mixtures consist of haploid (parental) cells which have not yet mated and of zygotes, some of which are in process of producing a first diploid bud. However, no free diploid cells (detached from the zygotes which produce them) are to be found as yet.

The following three operations are performed at this time:

A. A portion of each mating mixture is diluted and plated in Petri dishes on minimal medium (M.M.), where only prototrophic cells are able to proliferate. Therefore, each of the colonies formed on these plates represents the vegetative progeny (clone) produced by a zygote. The size of the colony indicates the normal or mutant (with respect to respiration) character of the clone, which is verified by spectroscopic examination.[8]

B. A large portion of each mating mixture is placed on sporulation medium (Sp.M.).

C. One or two drops of each mating mixture are inoculated into tubes of complete medium and incubated overnight, during which period additional zygotes and diploid cells are produced; the latter undergo a maximum of six generations. Thereafter, the cells are transferred to sporulation medium.

The asci produced in the few days following operations B and C are dissected, and ascospores are isolated in the microdissection chamber in droplets of complete medium, where they are allowed to give rise to haploid clones. These clones are plated out on complete medium. As in operation A, the size of the colonies formed on the plates is taken as an indication of the normal or mutant (respiratory) character of the clone, and this is verified by spectroscopic examination.[8] The presence of the different marker genes is tested by platings on appropriately supplemented synthetic media.

The results of the three operations described above are given in Figure 1 and may be summarized as follows:

A. The zygotes of the cross involving a neutral "petite" (cross $N \times n$, left side of Fig. 1), plated on minimal medium, give rise to a great majority of colonies of normal size. The percentage of small colonies is equal to the percentage of "petites" in parent-strain N, established by a control plating of the latter on complete medium (not shown in Fig. 1).

Under the same conditions, the zygotes of the cross involving a highly suppressive "petite" (cross $N \times s$, right side of Fig. 1) give the reverse picture: the great majority of colonies produced are of small size.

Spectroscopic examination of cultures derived from the large and from the small colonies of the two crosses confirms that the latter are composed of mutant (respiration-deficient) cells, the former of normal cells.

B. When the mating mixtures are placed on sporulation medium immediately after zygote formation, the types of asci produced by the two crosses are different in the following ways: (1) All, or almost all, asci of the cross involving a neutral "petite" ($N \times n$) contain four spores which give rise to clones that are normal with respect to respiration (asci 4:0). (2) All, or almost all, asci of the cross involving a highly suppressive "petite" contain, on the contrary, four spores which give rise to respiration-deficient clones (asci 0:4). Further crosses of segregants from these asci, thus far performed on a limited scale, show that all of them belong to the class of suppressive "petites."

In the asci of both crosses, the marker genes undergo 2:2 segregation.

C. If, prior to being placed on sporulation medium, the mating mixtures are subjected to a transfer, the asci of both crosses are of the 4:0 type, i.e., they contain

only spores which give rise to normal clones. Here again, the segregation of the marker genes is 2:2 in every ascus.

INTERPRETATION

I. *Cross $N \times n$.*—The fact that all four spores of the great majority (or all) of the asci of this cross give rise to normal clones indicates that the two parental strains are not differentiated by a single Mendelian gene. This observation corroborates the results of earlier work,[5] in which the cross $N \times n$ (involving a different "petite" strain) was followed by a series of four backcrosses to the mutant parent. It may be recalled that the results of these backcrosses were similar to those of the F_1, i.e., the backcross asci contained only spores giving rise to normal clones.[9] It was concluded that the respiratory deficiency is most probably not transmitted in a Mendelian fashion, and, instead, the following interpretation was suggested, which will be adopted here in view of the similarity of the present results with those previously published.

It is assumed that the normal yeast cell contains in its cytoplasm a particulate autoreproducing factor, to be referred to below as the "normal cytoplasmic factor," required for the synthesis of respiratory enzymes, and that the mutation $N \rightarrow n$ is the result of the loss of this factor or of its mutation to a functionally inactive form. When the two cell types (N and n) are crossed, zygote formation is accompanied by the mixture of the cytoplasms of the fusing cells. As a result, practically all diploid cells produced by the zygotes contain the normal cytoplasmic factor. Hence, plated on minimal medium (operation A), the zygotes give rise to colonies of normal size. The production on the plates of a few small colonies is ascribed to the presence in the mating mixture of some mutant zygotes resulting from copulations between cells of the mutant parent with the few similar cells ("petites") always present in populations of normal yeast. Finally, when the "hybrid" diploid cells sporulate, some particles of the normal factor are included in each of the spores. Each of these spores, therefore, gives rise to a normal clone.

If this interpretation is accepted, it appears that, from the point of view of the character considered (respiratory deficiency), the behavior of a neutral "petite" in a cross with normal yeast is that of a purely neutral element which contributes nothing to the zygote. This point is emphasized here because, as will be seen in the next paragraph, it is in this respect that neutral and suppressive "petites" are strikingly different.

II. *Cross $N \times s$.*—In interpreting the results of this cross, we shall begin by considering its first characteristic feature: the high proportion of small colonies produced on plating the mating mixture on minimal medium (operation A), i.e., the high proportion of zygotes giving rise to clones of mutant diploid cells. The simplest hypothesis which would account for this fact consists in ascribing these zygotes to preferential mating between cells of the mutant strain (s) and the few "petites" always present in the normal strain (N). However, experiments especially designed to this effect (pair matings in the microdissection chamber) definitely disprove this assumption. Therefore, the predominant occurrence of zygotes giving rise to mutant diploid clones must be regarded as the result of an *active process of suppression* by the mutant parent (s) of the normal cytoplasmic factor con-

tributed to the zygote by the normal parent (N). In other words, in contrast to neutral "petites," the suppressive "petites" may not be regarded as purely neutral elements. They contribute to the zygotes something which suppresses the perpetuation of the normal cytoplasmic factor contributed by the normal parent, by destroying it or by interfering with its multiplication or its distribution into the diploid buds. This something will be referred to as the "suppressive factor."

The production by the same cross of a few zygotes which give rise to normal diploid clones may be ascribed to the occurrence in the suppressive strain (s) of some neutral "petites" (n) which owe their origin to mutations $s \rightarrow n$ and which, in the cross with N, behave like the neutral "petites" discussed above. The occurrence of mutations $s \rightarrow n$ (which may consist in the loss or mutation of the suppressive factor) is confirmed by the isolation from suppressive clones of purely neutral subclones.

Turning now to the interpretation of the results of the analysis of the asci of the cross $N \times s$, it may be recalled that the facts to be accounted for are (a) the occurrence, incompatible with a Mendelian segregation in a diploid, of asci of both 4:0 and 0:4 types and (b) the dependence of the type of asci observed on the time at which sporulation is induced. The following interpretation is based on the assumptions that (a) the two types of asci are derived, respectively, from the two types of zygotes described above and (b) the peculiar time relationship is due to a delay in the effect of the suppressive factor, assumed to be cytoplasmic and autoreproducing.

The cross $N \times s$ produces a majority of zygotes containing the suppressive factor. If this factor immediately converted all these zygotes into "petites," their diploid vegetative progeny could not sporulate (because "petites" are unable to sporulate). It is therefore assumed that the effect of the suppressive factor is not immediate and that the diploid cells produced by this class of zygotes, placed on the sporulation medium immediately after their formation (operation B), are still able to sporulate, because they still contain the normal cytoplasmic factor or, at least, the preformed respiratory enzymes. However, all the ascospores thus produced carry the suppressive factor and therefore give rise to mutant clones. Under the same conditions, asci of the 4:0 type are rarely observed because of the rarity of the class of zygotes producing normal diploids.

When, on the other hand, prior to being placed on sporulation medium, the mating mixture is subjected to a transfer (operation C), the suppressive factor is granted the time to convert the diploid cells into "petites" unable to sporulate (the preformed enzymes must be "diluted" in the course of the cell multiplication which takes place during this period). Therefore, the only asci produced after the transfer are those derived from the zygotes which have received no suppressive factor, i.e., from the zygotes resulting from copulations of cells of the normal strain with the few neutral mutants contained in the suppressive strain. All the asci observed in this case are therefore of the 4:0 type.

The proposed hypothesis thus accounts, in a rather simple fashion, for the results of the experiments described thus far, including the peculiar time sequence in the appearance of the two types of asci. It is supported by the already mentioned isolation from suppressive strains of purely neutral subclones and by the evidence that all spores of the 0:4 asci give rise to suppressive clones. An additional problem is, however, posed by the existence of "petite" strains exhibiting various "degrees

of suppressiveness," i.e., of strains intermediate in their behavior between the two extreme types deliberately selected above for the description of the model experiment.

The occurrence of such strains can be demonstrated, and their degree of suppressiveness measured approximately, by the percentage of zygotes giving rise to mutant diploid clones following a cross with a normal strain (i.e., by the percentage of small colonies in operation A). If a number of "petite" strains are studied in this manner, it is found that they exhibit a variety of degrees of suppressiveness, ranging from 0 to nearly 100 per cent.[10]

The degrees of suppressiveness characteristic of different strains are relatively stable; they tend to be perpetuated as such in the course of vegetative reproduction. A decrease of suppressiveness can, however, occur spontaneously and can be experimentally induced. Thus a highly suppressive strain can eventually become neutral. Changes in the opposite direction either do not occur or are sufficiently rare to escape detection at the population level.

Concerning the significance of the different degrees of suppressiveness, two extreme hypotheses can be formulated:

1. There exist *at the cell level* only two types of "petites": a neutral and a completely suppressive type. Suppressive *strains* are mixed populations of the two cell types, coexisting in proportions which assume different values in different strains: the smaller the proportion of neutral "petites," the higher the degree of suppressiveness of the strain.

2. The cells of a suppressive strain (with the exception of the few neutral mutants present in the latter) all belong to a single type characterized by a certain *probability* (*i.e., efficiency*) *of suppression* which has different values in different strains. This implies that there may exist as many cell types as there are degrees of suppressiveness.

Experimental evidence bearing on these hypotheses and on the mode of action of the suppressive factor will be published shortly.

SUMMARY

The occurrence of two different classes of vegetative, respiration-deficient mutants of yeast is described. The behavior of the mutants of the two classes, referred to as "neutral 'petites'" and "suppressive 'petites,'" in crosses with normal yeast is illustrated by a model experiment.

* Guggenheim Fellow, 1952–1953; on leave from the University of Washington, Seattle.

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[8] The spectrum of "petite" yeast differs from that of normal yeast by the absence of the bands of cytochromes a and b (P. Slonimski and B. Ephrussi, *op. cit.*).

[9] Two further backcrosses gave similar results (B. Ephrussi and H. Hottinguer, unpublished).

[10] The degree of suppressiveness of a strain is measured by the percentage of zygotes giving rise to mutant clones following a cross with a normal strain *minus* the percentage of "petites" in the latter.

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*A CYTOGENETIC STUDY OF THE X CHROMOSOME OF DROSOPHILA BUSCKII AND ITS RELATION TO PHYLOGENY**

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Drosophila busckii, the only known representative of the subgenus *Dorsilopha*, has a number of genetic and cytologic peculiarities that shed special light on the probable phylogeny of the chromosomal groups in the genus *Drosophila*, no less on other problems of general genetic interest. Some of these features have been touched upon in earlier papers on comparative genetics,[1, 2] especially those on the cytogenetics of the Y chromosome of *D. busckii*.[3, 4] The present paper is concerned chiefly with new cytogenetic data that bear on the structure of the general chromosomal complement of this species, and especially upon the structure of the X chromosome.

According to current accounts, two chief chromosomal types of *D. busckii* exist in nature. In one there are four pairs of chromosomes: a pair of rod's, two pairs of V's, and a pair of dotlike chromosomes.[5] In the other chromosomal type there are said to be but three pairs of chromosomes, there being no dotlike elements. Furthermore, this dotless form is said to possess three morphologically different sorts of X chromosome, namely, a simple rod-shaped X,[1, 4, 6, 7] a rod-shaped X that terminates proximally in a spherical satellite,[8] and finally a J-shaped X chromosome.[9, 10]

In view of the conflicting accounts, the current investigation was undertaken with the aim of reinvestigating the chromosomal set of *D. busckii* and was carried along in three directions: (1) a cytological analysis of the mitotic and polytene chromosomes of *D. busckii* from geographically remote populations; (2) a cytogenetic analysis of certain mutant strains; and, finally, (3) an analysis of the homology of the X and Y chromosomes.

EXPERIMENTAL DATA AND RESULTS

The lines that were cytologically analyzed are listed in Table 1. In all lines the chromosomal complement was the same, consisting of three pairs of chromosomes. The rod-shaped pair is that of the sex chromosomes, and the two V-shaped pairs are autosomes. No evidence of gross dissimilarities in chromosomal complement was discerned in any of the strains.

The metaphase X chromosome has a constriction, evident in most preparations, that is located approximately one-third or one-fourth the length from the proximal end. Only occasionally is the metaphase X bent or flexed at this constriction.

TABLE 1

Drosophila busckii FROM DIFFERENT POPULATIONS*

Columbia, Missouri	1	1	Japan (different localities)	6	6
Verdi, Nevada	1	1	São Paulo, Brazil	1	1
Princeton, New Jersey	15	60	Edinburgh, Scotland	1	1
Oak Ridge, Tennessee	1	1	Belluno, Italy	1	1
Total				27	72

* The number of stocks from each locality in which mitotic and polytene chromosomes were studied are listed in the first and second columns of numbers respectively.

As a rule, the proximal end of X orients toward the center of the equatorial plate, and at anaphase it becomes more and more attenuated as it stretches poleward (Fig. 1, *A*, *B*, *C*).

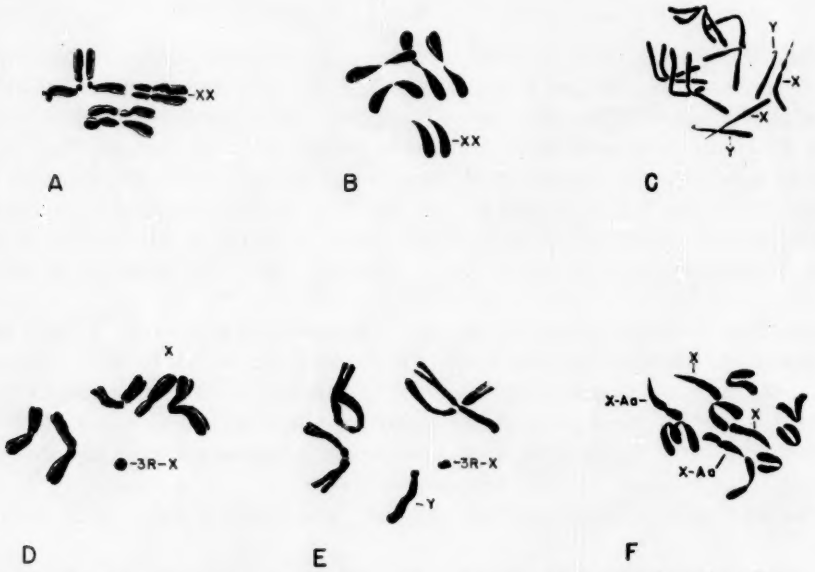

FIG. 1.—Chromosomal sets of *D. busckii*. *A*, metaphase from ganglial cell; *B*, metaphase from ovarial cell; *C*, anaphase from ganglial cell of male; *D*, female, and *E*, male, chromosomal complements from ganglial cells of *Cubitus interruptus* mutant; *F*, anaphase chromosomal set from ovarial cell of *Abnormal abdomen* mutant.

At mitotic prophase the X chromosome presents a very variable appearance. The constriction may stretch greatly, and then the long distal and short proximal parts of the X become widely separated. In extreme cases no microscopic connection can be observed between the distal and proximal lengths, and in such instances the short proximal part of X is easily mistaken for a dotlike chromosome.

The metaphase Y chromosome of the males in populations examined by the author shows a small knoblike thickening at the centromeric end. Between this knoblike thickening and the long distal part of the Y a slight constriction can some-

times be observed that is located approximately one-tenth or one-twelfth the length from the proximal end. In anaphase the Y has the appearance of a crochet hook, the crook of which is always directed to the pole (Fig. 1, *C*). Thus the Y chromosome of *D. buscckii* is a typical acrocentric chromosome, the centromere of which is located in the region of the nearly terminal constriction. In none of the strains investigated is the mitotic Y a simple rod-shaped element as has been claimed.[10] As shown in previous investigations,[4] the length of the Y up to the knoblike thickening represents the "left" arm and consists of heterochromatin. The small proximal knoblike ending represents the euchromatic "right" arm, which is the genetically active part of this chromosome.

The long V-shaped chromosomes of metaphase are the second and third pairs of autosomes. In metaphase and late prophase of ganglion cells these pairs can be easily distinguished from each other because one of them has only one constriction, namely, the centromeric, while the other pair has two constrictions that set limits to an oval-shaped block in the center of the chromosome. One of these constrictions is centromeric, the other secondary and of an unknown role. In ovarial cells of females, only primary constrictions can be observed in both pairs of chromosomes.

In salivary gland cells of females, five long and one short euchromatic elements are present. Of these, four of the long limbs represent the right and left arms of the second and third autosomes. Very often the left and the right arms of the corresponding autosomes remain connected by their proximal ends in squash preparations, forming uninterrupted strands of the whole chromosomes. Sometimes, in the proximal region of each, where the arms are connected, a constriction is observed, the length and the depth of which depend upon the degree to which the chromosomes are stretched. These constrictions are achromatic and correspond to the regions of the centromeres. The proximal ends of the arms adjacent to the centromeric regions are somewhat thicker than the distal parts of the chromosomes, perhaps due to a weakening of the conjugation of chromonemata. The fifth long limb and the short euchromatic element constitute the polytene X chromosome. Identification of these elements as parts of the X chromosome is easily made, owing to their univalent structure in males and their attachment to the nucleolus. In the polytene X the nucleolus organizer, hence the nucleolus, lies between the long limb and the short euchromatic element. When slides are made, the short euchromatic element is easily deformed, and it therefore often appears as a polymorphic body. The deformability of the short element is correlated with the heterochromatization of its distal end which attaches it to the nucleolus. In all 72 lines the nature of the nucleolar attachment of both long and short euchromatic elements is the same, the X chromosomes all being structurally alike (Fig. 2).

In the salivary gland cells of males, in addition to the euchromatic elements so far described, there is a second short element, namely, the euchromatic part of the Y chromosome (Fig. 2). As shown in a previous investigation,[4] this euchromatic part of Y corresponds to the knoblike thickening of the mitotic chromosome. Usually the short elements of the X and Y chromosomes are connected by their proximal ends.

Although it seemed natural to suppose[1, 4, 7, 9, 11] that the short element of the X represented the right arm of X and that the centromere was located in or near

the nucleolus organizer, neither conclusion is correct. This has been demonstrated by means of chromosomal rearrangements. The polytene chromosomes of the dominant *Cubitus interruptus* (*Ci*, 585) mutant, which phenotypically is similar to the corresponding mutant of *D. melanogaster*, display a complicated rearrangement that involves X, 2R, 3L, and 3R. In this case the break in X is located in the distal part of the short euchromatic element, namely, in the right half of subdivision 20*A* (Fig. 2, *Ci*). 2R is broken at the demarcation of divisions 22 and 23. 3L is broken at approximately division 13. Finally, 3R has two breaks: one end of division 2, the other approximately in division 6.[12] The order of union among the fragments is as follows: (1) the short distal part of 3R, consisting approximately of 12 disks, is connected with the proximal part of the short euchromatic element of the X chromosome; (2) the distal part of 3L is translocated onto the proximal part of 3R; (3) the long distal part of 2R is translocated to the middle of 3R (which arose as a result of breaks in divisions 2 and 6), and the middle part of 3R is connected with the proximal part of 3L; finally, (4) the entire part of the long euchromatic element of X, together with the nucleolus organizer and small distal part of the short euchromatic element, is translocated onto the proximal part of 2R.

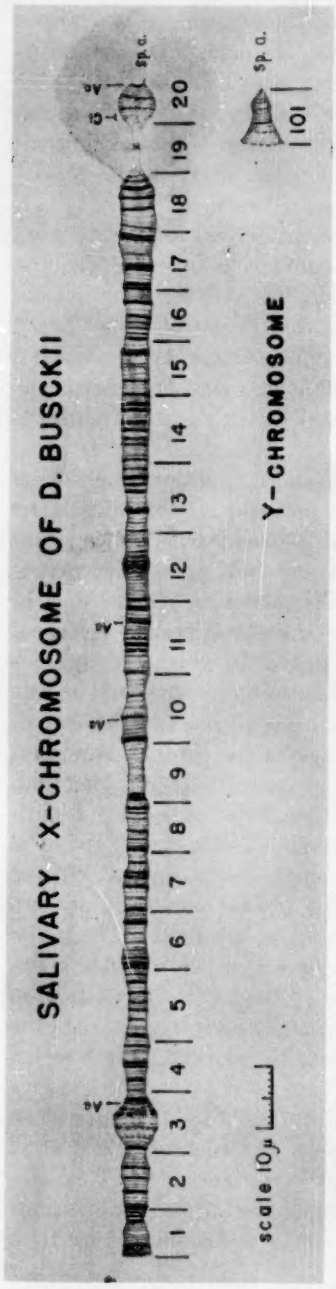

FIG. 2.—The symbols *Aa* and *Ci* indicate the regions of breakage in the X chromosomes of the mutants *Abnormal abdomen* and *Cubitus interruptus*.

The short chromosome that arose by the union of the distal part of 3R and the proximal part of the short euchromatic element of X is of special interest. In the polytenes of mutant males it can ordinarily be seen close to the euchromatic part of the Y chromosome; indeed, it may conjugate proximally with this portion of Y. But most significantly, it is wholly independent of the nucleolus as well as of all chromosomes other than Y.

Ganglial and ovarial complements of females heterozygous for *Cubitus interruptus* display three pairs of chromosomes: two V-shaped pairs and a pair of very unequal chromosomes. The heteromorphic pair consists of a long rod-shaped chromosome, the normal X, and a short dotlike chromosome that tends to be located adjacent to the proximal end of

X. *Cubitus interruptus* males have identical sets of V-shaped chromosomes, a normal Y, and the new dotlike chromosome (Fig. 1, *D* and *E*).

Cytogenetic analysis shows that the factor, or position effect, giving the *Cubitus interruptus* phenotype is located in the new short chromosome, and thus the short chromosome is easily followed in genetic crosses. When the mutant *Cubitus interruptus* male is crossed by a normal female, *Cubitus interruptus* behaves as a dominant sex-linked factor primarily opposed to Y in its segregation. But in crosses where the X chromosomes of females are marked with a recessive gene, some 1.3 per cent exceptional males appear in addition to the expected regular classes of females and males. For example, crosses of *yellow* ♀♀ × *Cubitus interruptus* ♂♂ gave 897 *Ci* ♀♀, 808 *y* ♂♂, and 22 (1.3 per cent) *y;Ci* ♂♂ in a total of 1,727 offspring.

These *y;Ci* exceptional males are peculiar by token of the fact that they inherit X-chromosomal factors from both their mothers and their fathers. Both genetic and cytologic analyses show these males to possess a normal complement of autosomes, a normal X chromosome, and a normal Y, as well as a supernumerary element—the short *Ci*-bearing chromosome. When crossed by normal females, these exceptional males give a remarkable progeny, for *Cubitus interruptus* is now inherited as though it were a dominant autosomal factor. Thus crosses of *white* ♀♀ × *yellow; Cubitus interruptus* ♂♂ gave 686 phenotypically normal ♀♀, 383 *Ci* ♀♀, 434 *w* ♂♂, and 298 *w;Ci* ♂♂ in a total of 1,801 offspring. The lower number of *Ci* flies in both sexes is of no special significance, evidently being due to their lower viability.

This surprising segregation is due to the fact that the new short chromosome now behaves as a simple duplication, randomly segregating with respect to both X and Y, which behave normally. Thus the occurrence of a high frequency of *Cubitus interruptus* offspring, the normality of the expression of this phenotype in them, and the independence of the short chromosome at reduction collectively indicate that the new short chromosome possesses its own centromere.

Now the length of this short dotlike chromosome in somatic cells is approximately equal to half the length of the proximal part of the normal metaphase X chromosome, i.e., about one-sixth or one-eighth the total length of the X. Although composed of a small distal part of 3R and a portion of the proximal region of X, it is not possible to say exactly what fraction of the total mitotic length of the short chromosome is contributed by each of these. Muller and Painter[13] have shown that the small portion of the X chromosome of *D. melanogaster* extending from the distal tip to the region of *white* is cytologically unresolvable at mitotic metaphase. The section of 3R involved in this new short chromosome of *D. busckii* has approximately only half as many bands as the tip-*white* region of the X of *D. melanogaster*. Very likely 3R contributes insignificantly to the short *Ci*-bearing chromosome, and the greater bulk of this chromosome is very probably made up of the proximal part of the X. However, the apical euchromatic X-chromosomal portion of the new 3R-X fusion chromosome is itself of comparatively small size. Therefore, the proximal heterochromatin of X must make up the bulk of the short dotlike chromosome. In polytenes the terminal portion of 3R forms the distal tip of the 3R-X compound. For this reason it is very likely that the centromere of the small chromosome lies somewhere in its part rightmost, namely, in the heterochromatic portion of the chromosome.

Similar conclusions may be drawn from rearrangements occurring in the dominant mutant *Abnormal abdomen* (*Aa*, 797). In polytenes of females heterozygous for *Abnormal abdomen*, the X gives synaptic configurations typical for heterozygous overlapping inversions. The *Abnormal abdomen* aberration involves four breaks in X: one at the end of division 3, one in 10*B*, a third at the end of 11*C*, and the fourth in 20*D* beyond the proximal end of the short euchromatic element (Fig. 2, *Aa*). The order of union among these five fragments is (1) distal fragment, (2) inverted fragment 10*B* to 11*C*, (3) inverted fragment 11*C* to 20*D*, (4) fragment 3 to 10*B* in original order, and, finally, (5) the centromeric part, 20*D*.

Despite the fact that in this chromosome the greater proximal part of X has been involved and transposed into the distal half, including as it does seven of the sections of the long euchromatic element, the nucleolus region, and practically all of the short euchromatic element, the rearranged X in metaphase and anaphase is still rod-shaped and still has a nearly terminal centromere (Fig. 1, *F*). The centromeric part of a normal X, then, must be so small as to be invisible microscopically and corresponds to only a small apical portion of the proximal third (or fourth) of the normal X chromosome.

The results of the analysis of *Cubitus interruptus* and *Abnormal abdomen* mutants agree in placing the centromere of the normal X chromosome of *D. busckii* very near the tip of the proximal end, at the right end of the short euchromatic element. In other words, the pronounced constriction at the apical third or fourth of the metaphase X is not centromeric, nor is the short euchromatic element a right arm of the polytene X chromosome (Figs. 2, 3).

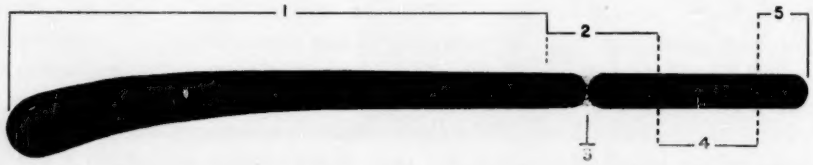

FIG. 3.—Diagram of the mitotic X chromosome of *D. busckii*: *1*, distal part corresponding to the long euchromatic element of the polytene chromosome; *2*, heterochromatic part with constriction (*3*) corresponding to the nucleolar region of the polytene X chromosome; *4*, region corresponding to the short euchromatic element of the polytene X chromosome; *5*, proximal heterochromatic part with the location of the centromere.

On the basis of earlier studies of the polytene and mitotic chromosomes of males of *D. busckii*, I suggested a homology of the polytene euchromatic element in Y with the short euchromatic element of X.[4] Although the basis for this suggestion seemed convincing, final proof nevertheless required a genetic demonstration of the validity of the conclusion.

Now, if a genetically active region in X is homologous with the euchromatic part of Y, then allelic genes should occur in these chromosomes. Presumably, mutations of some of these genes may be expected among the progeny of flies involved in large-scale irradiation experiments. If a recessive mutant occurs in the supposedly homologous region or regions of X and then is made homozygous within a line, males and females of this strain should be different in phenotype. In the case of a lethal mutation, homozygous females could not occur but males bearing such a recessive lethal should be fully viable.

All together, 1,598 lines were established, each line obtaining an X-rayed X chromosome. Five of these lines proved to have recessive X-chromosomal mutations for which a normal allele occurred in the Y chromosome. Of the five lines, three contained different lethal mutations in X and two possessed allelic mutants with visible phenotypes. By adding different fragments of the Y chromosome to the chromosomal sets of these females, it was possible to prove that the normal alleles for each of these X-chromosomal mutant genes are located in the right arm of the Y chromosome, which, it will be recalled, is a small euchromatic element. Finally, by adding the short chromosomal duplication from the *Cubitus interruptus* line to the female karyotype of these five mutant strains, it was proved that the hereditary factors involved in the induced mutations are all located in the short euchromatic element of the polytene X chromosome. Thus the homology of the short proximal element in X with the euchromatic element in Y may now be taken as proved.

The question arises as to the evolutionary origin of the euchromatic element common to X and Y. Both the present author[1, 4] and Wharton,[7] as well as some others,[9] have suggested that the short euchromatic element or the X represents the microchromosome (dot) that is present in many species of *Drosophila* but is absent in *D. busckii*. Such a conclusion is reasonable on the basis of the comparative morphology of both polytene and mitotic complements, but to date no genetic evidence has been brought forth for this belief.

It is known that *Cubitus interruptus* is a characteristic mutation of the fourth (dot) chromosome of *D. melanogaster*. The hereditary factor in *D. busckii* described by us as *Cubitus interruptus* is located in the derived short or dotlike chromosome that is made up of the tip of 3R and the proximal part of the short euchromatic element of the polytene X chromosome. Some evidence suggests that the factor *Cubitus interruptus* resides in the X-chromosomal part of this 3R-X compound. To date, this is the only genetic evidence of at least a partial homology of this small euchromatic block in X and Y with the microchromosomes.

It would seem, then, that in the process of evolution translocations of the microchromosomes took place stepwise to both X and Y or to one of the sex chromosomes only. In the latter case the euchromatin of the microchromosome may have been transferred to the other sex chromosome by crossing over. As is the case in species with free microchromosomes, then, these euchromatic elements are also present in equal dose in both sexes of *D. busckii*.[4]

In almost all species of *Drosophila* studied to date[7, 9, 14] there is a short euchromatic element among the polytene chromosomes. However, in about a quarter of these species, just as in *D. busckii*, there are no dotlike chromosomes or microchromosomes present in either mitotic or meiotic nuclei. Comparative cytological analysis of the chromosomal complements has led to the supposition that in some of these dotless species, in which there has been no reduction in number of centromeres, the microchromosomes have acquired heterochromatin from other chromosomes during the process of species differentiation. *Drosophila ananassae* is a good example of such a change in karyotype. Its microchromosomes, as shown both cytologically and genetically, have acquired most of the heterochromatic region of the X chromosome, including the locus of the gene *bobbed* as well as the nucleolus organizer.[15] As to the others of this dotless group of species, in which the "loss" of the dots has been accompanied by a reduction in number of centromeres, com-

parative cytological analysis has led to the view that in these cases the microchromosomes have become parts of the macrochromosomal elements (either by translocation or by centric fusion with major chromosomes). Thus it is held that in all these cases the microchromosomes preserve their individuality in nuclei with polytene chromosomes because they have been included in the heterochromatic regions of macrochromosomes. This general view is widely accepted and is no doubt correct, but until now it has not been supported by any direct genetic evidence. The data described above demonstrate a direct homology of the short euchromatic elements within the X and Y chromosomes of *D. busckii* with the microchromosomes of *D. melanogaster*. So far this is the only cytogenetic proof of a case of intercalation of the dot chromosomes within the structure of major chromosomes, with a consequent reduction in the number of centromeres.

SUMMARY

1. The normal mitotic chromosome complement of *D. busckii*, so far as is now known, is of one sort only. It contains three pairs of chromosomes: a pair of rod-shaped sex chromosomes and two pairs of nearly equal-sized V-shaped autosomes. The X chromosome has a prominent achromatic constriction that corresponds with the nucleolus organizer and which lies approximately one-third (or one-fourth) the length of the metaphase chromosome from the proximal end. The centromere of the mitotic X is very nearly at the tip of the proximal end, and perhaps this chromosome is telocentric (Fig. 3). The Y chromosome has a small knoblike thickening at the centromeric end. The primary constriction can be observed between this knoblike thickening and the long distal part of the Y. Thus this chromosome is acrocentric, or J-shaped.

2. The short euchromatic element of the polytene X chromosome represents the proximal part of the X, and it is now proved that this element is homologous with the euchromatic portion of the polytene Y chromosome, that corresponds to the proximal knoblike thickening, or right arm, of Y.

3. The homologous euchromatic portions of X and Y of *D. busckii* are, in turn, homologous with the microchromosomes of other species of *Drosophila*.

4. The data given here concerning these homologies provide the first cytogenetic evidence for the supposition that the microchromosomes of other dotless species may have become parts of macrochromosomal elements during the process of species differentiation.

* This investigation was supported in part by a grant from the Rockefeller Foundation to Princeton University and in part by a research grant (G-3934) from the National Institutes of Health, Public Health Service, to the University of Rochester. I am very grateful to the authorities of these institutions for the provision of facilities and for the hospitality they have shown me.

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[11] A. H. Sturtevant, "The Classification of the Genus *Drosophila*, with Description of Nine New Species," *Univ. Texas Publ.*, No. 4213, pp. 5–66, 1942.

[12] The regions of the breaks in the X were determined from the map of this chromosome prepared by J. Krivshenko (to be published). This map consists of 20 divisions, each of which is divided into 4 subdivisions (*A*, *B*, *C*, *D*). The gross banding patterns of this new map are represented in Fig. 2. The break-point regions in the autosomes were determined from Sirotina's map (*op. cit.*). Regrettably the latter map is very schematic, and the regions of the breaks are only approximate.

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*DYSTROPHIA MUSCULARIS: A HEREDITARY PRIMARY MYOPATHY IN THE HOUSE MOUSE**

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Communicated by C. C. Little, October 7, 1955

Several mutations characterized by neuromuscular symptomatology, either of unknown etiology or associated with primary neuropathology, have been reported in mammals.[1] Although primary involvement of striated muscle has frequently been described in humans,[2] reference to spontaneous myopathy in animals is rare. The present communication describes a myopathic mutation in mice, apparently the first recorded instance of primary pathological involvement of muscular tissue in an experimental animal of known genetic background. In view of the striking similarity between the lesions in this mutant and those found in human muscular

dystrophy, it appears that the condition reported here will provide a useful tool for research in the field of muscular disease.

The anomaly, characterized by progressive ataxia, atrophy, and paralysis, first appeared in 1951 in Dr. Elizabeth S. Russell's colony of inbred strain 129 mice at the Roscoe B. Jackson Memorial Laboratory. The hereditary pattern of the defect suggests the presence of a recessive autosomal mutation. Since the histological findings in the muscular and neural tissues are similar to those found in the muscular dystrophies, it is suggested that the mutation be called *dystrophia muscularis* and be designated by the symbol *dy*.

1. *Clinical Observations.*—The affected animal is lighter in weight and smaller in size than normal strain 129 mice of the same age. This observation has been confirmed by data which establish that the dystrophic mouse is consistently lighter than its normal litter mates at 2, 3, 4, and 5 weeks of age, with subnormal weight continuing throughout its life-span.

The hind limbs flex spasmodically when the animal is suspended by the tail (Fig. 1). In walking, the hind limbs are dragged behind with muscles in flaccid extension (Fig. 2). When placed on a table for observation, the dystrophic mouse frequently sits in a tensed position and convulsively nods its head up and down in a 90° arc.

Clinical manifestation of the disease is first recognizable at $3^1/_2$ weeks of age, when signs of ataxia with occasional unilateral paresis of the hind limbs are observed. Subsequently bilateral paresis sets in. At approximately 8 weeks the animal's cachetic appearance is enhanced by a thinning and ruffling of the fur. As the paralysis becomes established, muscular atrophy appears, proceeding from the hind quarters to the axial and forelimb musculature and resulting in a distinct ky-

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FIG. 1.—Photograph of a $4^1/_2$-month-old normal strain 129 female (*left*) and a $4^1/_2$-month-old dystrophic strain 129 female suspended by their tails. The normal animal clutches wildly at the air with its hind feet. The dystrophic animal has reached the terminal stages of the condition, where it suffers complete loss of locomotor control in the hind limbs, which are fairly permanently extended at an abnormal angle from the body.

FIG. 2.—Photograph of a 2-month-old dystrophic strain 129 male. The atrophied hind limbs are seen in flaccid extension. The characteristic kyphosis, which usually appears at this stage, is already marked.

FIG. 3.—Photomicrograph of a cross-section of thigh musculature from a 2-month-old normal strain 129 female. Note the relative area and the polygonal contour of the fibers, the peripheral location of their nuclei, and the amount of interstitial tissue forming the endomysium. (Hematoxylin and eosin. ×200.)

FIG. 4.—Photomicrograph of a cross-section of thigh musculature from a 2-month-old dystrophic strain 129 female. Note the relatively small area and the rounded contour of the fibers, the central location of many of the nuclei, and the increased amount of interstitial tissue. The peripheral nerve seen in transverse section in the lower right-hand corner contains the uncollapsed neurolemma, with apparently normal sheath of Schwann cell nuclei, indicative of normal nerve fibers. (Hematoxylin and eosin. ×200.)

FIG. 5.—Photomicrograph of a longitudinal section of the biceps femoris of a 3-month-old normal strain 129 female. Note the even width of the fibers, the relatively small amount of space between the individual fibers, and the relatively sparse distribution of sarcolemmal nuclei. (Hematoxylin and eosin. ×100.)

FIG. 6.—Photomicrograph of a longitudinal section of the biceps femoris of a 3-month-old dystrophic strain 129 female. Note the varying width of the fibers, the increased amount of space between the fibers occupied by an increased amount of interstitial tissue, and the marked proliferation of nuclei both within and between the fibers. The cytoplasm of the fiber in the upper left-hand corner is in an advanced stage of degeneration. Many of its nuclei have become pyknotic. (Hematoxylin and eosin. ×100.)

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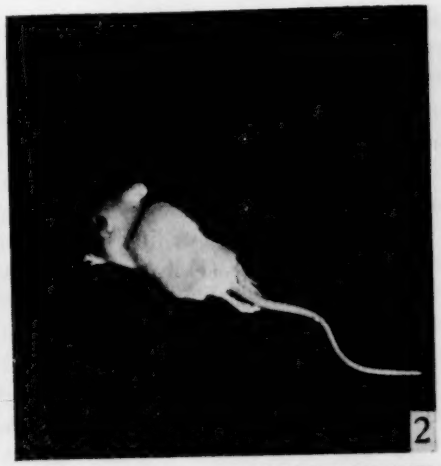
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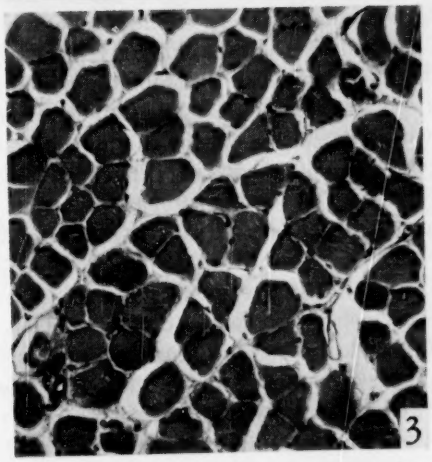
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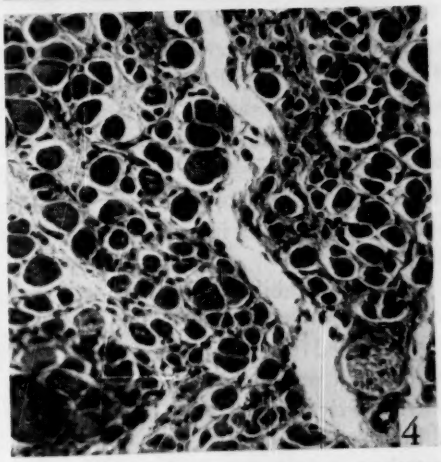
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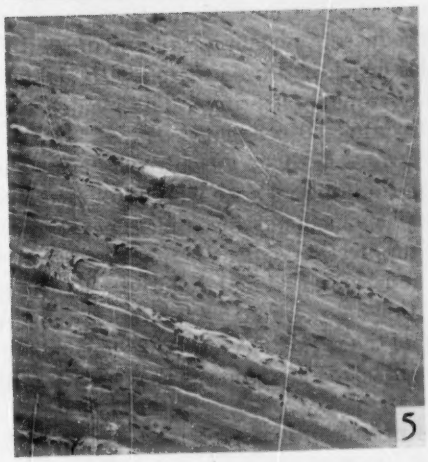
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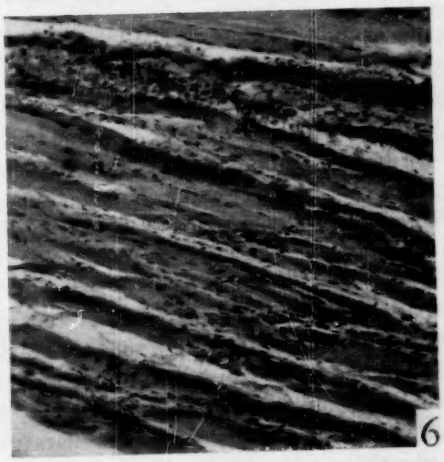
6

phosis. In the terminal stages, functional control of the hind limbs is completely lost, locomotion being achieved entirely by means of the atrophied forelimbs.

Death may occur at various points in the progression of the disease. Some of the smallest animals, suspected of *dystrophia*, die prior to weaning, before recognition of the condition can be confirmed. Most of the remainder die between 1 and 6 months of age, although several badly affected females have lived to 8 and 9 months. The direct cause of death has not been definitely ascertained.

2. *Genetics.*—The hereditary etiology of *dystrophia muscularis* was inferred from the following facts: (1) its appearance is exclusive within one of the several strain 129 colonies maintained at the laboratory; (2) its expression within the inbred strain is selective, involving only a limited number of individuals in an affected litter; (3) its incidence is frequently higher among the offspring of siblings of dystrophic animals than among those of other matings; (4) its transmission is effected even when isolation from abnormal animals and from those exposed to the condition has been maintained for as long as two generations.

Breeding data have been compiled for 2,950 animals. The combined general incidence of the defect within all portions of the pedigree of the colony is 6 per cent. The combined specific incidence among the offspring of all dystrophic-producing matings is 13 per cent. The general and specific incidences vary respectively, from 1 to 9 per cent and from 6 to 20 per cent within different portions of the pedigree. Such wide variation may be due to the impossibility of recognizing carriers and the ensuing probability of loss of the mutation. The incidence of *dystrophia muscularis* among the offspring in individual matings producing the defect ranges from 3 to 37 per cent.

Physical disability prevents the successful mating of dystrophic animals. Histological preparations of the gonads reveal normal morphology. Since the statistics compiled from the breeding data did not provide satisfactorily conclusive genetic evidence, it was decided to employ the technique of ovarian transplantation.[3] The ovaries of dystrophic females were transplanted unilaterally to the ovarian capsules of bilaterally ovariectomized normal hosts of the same strain, which were then mated to normal males. Three normal host females, whose donated ovaries originally came from two dystrophic females, produced a phenotypically unaffected F_1 generation. Each of the thirteen F_1 females which had been mated with eight F_1 males, all presumably carriers, produced dystrophic offspring. The incidence of the defect among the 117 F_2 offspring born to date is 21 per cent.

In light of the above evidence, it seems probable that *dystrophia muscularis* is caused by the direct action of a single mutant autosomal gene whose full recessive expression of 25 per cent is obscured by the phenomenon of incomplete penetrance, a masking effect due to the modification or suppression of gene action produced by interaction of the particular environment and the individual genotype. Further experiments are being carried out to test this assumption. Attempts to collect linkage data and to ascertain the expressivity of the mutation on the different genetic backgrounds of other inbred strains of mice will be made as soon as they are deemed propitious and practicable.

3. *Pathology.*—Histological preparations of neural and muscular tissues from dystrophic and control animals included all levels of the central nervous system

stained by the Swank and Davenport modification of the Marchi technique, the lumbosacral spinal cord prepared by Nissl and silver methods, the sciatic nerve stained with osmium tetroxide, and the hind-limb musculature and related nerves prepared with hematoxylin and eosin and the Romanes silver chloride method.

No demonstrable pathology was found in any part of the examined neural tissue. However, study of the muscle preparations revealed proliferation of sarcolemmal nuclei, increase in the amount of interstitial tissue, and size variations among individual fibers (Figs. 3–6). A mixed distribution of pathological and apparently normal fibers occurs. Fibers which are otherwise normal sometimes contain long chains of nuclei which, in cross-section, are noted centrally, in marked contrast to the characteristic peripheral position. In areas where fairly complete degeneration of the fiber has taken place, clumps and rows of pyknotic nuclei remain. Instead of presenting a typical polygonal contour, the affected fiber as seen in transverse section appears rounded, with a cross-sectional area which is considerably smaller than that of the normal fiber. Occasional splitting of fibers to give two or more daughter-fibers, all contained within a single endomysial tube, has been observed. The marked increase in the amount of connective tissue is readily noted in both transverse and longitudinal sections. In several instances, rows of fat cells were found between the fibers. The degree of fatty replacement in the final stage has yet to be determined. Regenerating fibers have not been observed. Peripheral nerves occurring within the affected muscle, as well as the sciatic nerves of affected limbs, were ostensibly normal.

The above findings are in close agreement with the pathological criteria regarded as characteristic of the muscular dystrophies (cf. Adams, Denny-Brown, and Pearson[2]). A more precise definition of dystrophy in the mouse, as well as its comparison with the various histologically distinct types of muscular dystrophy recognized in human myopathy, must await more comprehensive histopathological studies.

4. *Summary.*—A primary myopathic mutation, characterized by progressive ataxia, atrophy, and paralysis, occurred in a colony of inbred strain 129 mice in 1951. It has been suggested that the hereditary anomaly be referred to as *dystrophia muscularis*, designated by the symbol *dy*.

Clinical manifestations include consistent lightness of body weight, general atrophy of the axial and limb muscles, convulsive nodding of the head, kyphosis, paresis accompanied by spasmodic flexion and flaccid extension of the hind limbs, eventual complete loss of locomotor function in that region, and premature death.

Breeding data from the pedigree of the strain 129 colony and from ovarian transplantation experiments are interpreted as indicating the presence of a single autosomal gene whose full recessive expression of 25 per cent is obscured to a maximal average of 21 per cent.

Microscopic study revealed the pathological involvement of muscular tissue and the absence of ostensible lesions of central as well as of peripheral nervous tissue which are characteristic of the recognized histopathological syndrome of muscular dystrophy.

The mutant should provide a useful tool for research, since it is apparently the first experimental animal of known genetic background to present a myopathy similar to that found in the human muscular dystrophies.

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*RECOMBINATION INCREASE DUE TO HETEROLOGOUS INVERSIONS AND THE RELATION TO CYTOLOGICAL LENGTH**

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Various properties of the extreme left end of the X chromosome of *Drosophila melanogaster* make the region interesting for studies of the relationship of crossing over to chromatin. Among such properties is a disparity in the distances between loci as measured by the counting of bands in preparations of salivary gland chromosomes and the purely genetic distances given by linkage values. Thus the two short crossover intervals between *yellow* and *white* and between *white* and *split* are ordinarily equal; the standard linkage map gives them as 1.5 units each.[1] For comparison, the actual physical relations of these genes have been rather precisely defined by the cytological map of Bridges (1938); the number of bands from *yellow* to *white* (98) is approximately 20 times as great as the number (5) from *white* to *split*. One can, if he prefers, measure these distances in microns along the salivary gland chromosomes, but this is somewhat less objective, due to differential stretching. On Bridges' map,[2] however, the relative distances in this region remain in the same proportion no matter which cytological unit is used to measure separation, that is, banding or actual average distances along stretched chromosomes, and the disparity still holds—while the standard linkage values for the two subregions are 1:1, the cytological distances are as 20:1.

If the assumption is made that the structural relations are the same in the crossing-over chromosomes of the oöcytes as they are in the chromosomes of the somatic cells of the salivary gland, then it follows that there is 20 times as much crossing over per band in the *white-split* subregion as there is in the *yellow-white* subregion. (This is not meant to imply that crossing over necessarily occurs within the band.) The marked differential of 20 to 1 provides a convenient handle for experimentation, for, if the crossover values in the X can be changed by some agent external to it, then it can be determined whether the new values are approxi-

mately equal to each other or whether they become markedly unequal and are related (or are not related) to the difference in cytological length. In this connection it might be contended that crossing over is a direct function of length per se along the chromosomes and that the regional differences at the time of crossing over are not like those on the cytological map. Presumably there is less ground for believing that relative length as opposed to banding remains constant; of course, neither condition has been demonstrated. But however these matters eventuate, it is of interest to compare recombination changes in this region with the cytological map as it now stands.

The extreme left end of the X appears rather resistant to the usual agents which affect crossing over, such as temperature, X-rays, etc. However, the presence of heterozygous inversions in the autosomes has been shown greatly to increase recombination for the region. The presence of such heterologous inversions results in generalized increase in crossing over in noninverted pairs of the chromosome complement. These changes occur in a rather constant over-all pattern which displays some similarity to the pattern produced by other agents affecting crossing over (but there are differences). With heterologous inversions very large increases are shown near the centromere and typically also at free ends of the chromosome limbs; intermediate regions show increases, but these are less marked. (For a review see Schultz and Redfield.[3]) The problem of second-order differences has had little attention, but scattered bits of evidence, such as the exceptional decreases for the right end of III appearing in data of Steinberg and Fraser,[4] suggest that there is considerable variation within the pattern. It seemed desirable to set up a controlled experiment specifically to test the possible differential sensitivity, per unit of physical length, of the two small adjacent sections *yellow-white* and *white-split* to the booster effect on crossing over produced by simple inversions. The results have been presented in preliminary form elsewhere.[5]

Materials and Results.—Crossing over was measured in sister females of the composition *y* w^{51a}/*spl* of four types with respect to the inversions which they contained: those without inversions, those heterozygous for the left and right *Curly* inversions of II, those heterozygous for the left and right *Payne* inversions of III, and those simultaneously heterozygous for all four *Curly* and *Payne* inversions. The stocks used directly to derive the females, namely, *y* w^{51a}; *Cy*, sp^2/+ and *spl*; *Payne*, *Dfd ca*/+ (both continued by mating noninversion females by inversion males), were established from the highly inbred "p-1 Oregon-R" basic line of this laboratory; this made it reasonably certain that the chromosomes of tested females were alike except for the chromosomes containing the inversions. *White*^{51a} is a new occurrence of *white* which is phenotypically and cytologically indistinguishable from the original *white*. The structural picture of *white*^{51a} and the proper presence of the inversions were checked by the concurrent cytological study of Schultz and Hungerford[6] on inversion effects on patterns of pairing in salivary gland chromosomes of this same material.

All cultures and vials were kept at 25° C. Each virgin female from the early hatch of well-nourished parents was mated in a vial for one day with 10 tester males from y^2 *su-*w^a w^a *spl* stock (used instead of *y w spl* to insure proper fertilization of the non*yellow* females and to detect nondisjunction). She was then put with her males into a culture bottle, where she remained 5 days, and then was subcultured

for 5 days more. The number of such females per type varied from 13 for subcultures with *Curly* to 19 for the cultures with both *Curly* and *Payne*. Yields were good for all types; they varied from 205 per control female for subcultures to 237 per *Curly* female for first cultures.

The raw data and the crossover values with their differences are given in Table 1. The results are simple and consistent. It may be noted that crossing over without inversions is definitely below the expected standard value of 1.5 for each region, reaching only 0.4 and 0.5 for first cultures and 0.8 for both regions for subcultures. (Standard values are, of course, lumped values which presumably include data

TABLE 1

CROSSING OVER IN THE REGIONS y-w^{51a} AND w^{51a}-*spl* IN $y\ w^{51a}/spl$ FEMALES WITH INVERSIONS INDICATED*

INVERSIONS	NONCROSSOVERS	SINGLE CROSSOVERS y-w^{51a}	SINGLE CROSSOVERS w^{51a}-*spl*	CROSSOVER VALUES y-w^{51a}	CROSSOVER VALUES w^{51a}-*spl*	DIFFERENCE
Age 2–6 days:						
None	2,119 + 2,101	17 + 5	6 + 11	0.5 ± 0.11	0.4 ± 0.10	0.1 ± 0.15
Cy	1,585 + 1,658	21 + 16	18 + 24	1.1 ± 0.18	1.3 ± 0.20	−0.2 ± 0.27
Payne	1,800 + 1,716	22 + 29	18 + 22	1.4 ± 0.20	1.1 ± 0.17	0.3 ± 0.26
Cy; Payne	1,932 + 2,079	61 + 66	41 + 63	3.0 ± 0.26	2.5 ± 0.24	0.5 ± 0.35
Age 7–11 days:						
None	1,841 + 1,785	16 + 15	11 + 20	0.8 ± 0.15	0.8 ± 0.15	0 ± 0.21
Cy†	1,462 + 1,508	19 + 24	18 + 26	1.4 ± 0.21	1.5 ± 0.21	0.1 ± 0.30
Payne	1,503 + 1,497	27 + 24	22 + 29	1.6 ± 0.23	1.6 ± 0.23	0 ± 0.33
Cy; Payne	1,970 + 2,057	79 + 81	61 + 60	3.7 ± 0.29	2.8 ± 0.25	0.9 ± 0.38

* In each pair of contrary classes the class with *yellow* is given first. Crossover percentages and the differences are given with their standard errors.

† Also one y F_1 male, presumably a double crossover, which proved sterile.

involving undetected heterologous inversions.) Within an age type, for these females lacking inversions, there is no statistically significant difference in crossing over between the two regions y-w^{51a} and w^{51a}-*spl*. In the females with *Curly* inversions, crossing over is significantly increased for both first and second cultures, the increase being approximately equal in each case for the two regions. Similar practically equal increases are produced in the presence of the *Payne* inversions. With both *Curly* and *Payne* inversions the increases are even more marked, but they still show no significant difference between the two regions. In no case for a given type is there a detectable difference between the increases for y-w^{51a} and w^{51a}-*spl*.

Just how great these changes are is shown in Table 2, which gives the ratios of crossing over in each region for each inversion type to crossing over without the inversions. Thus, with due regard to age of the mothers, the *Curly* and *Payne* inversions separately increase crossing over 2 to 3 times the value in the absence of

TABLE 2

INCREASES PRODUCED BY INVERSIONS EXPRESSED AS RATIOS OF CROSSING OVER IN $y\ w^{51a}/spl$ FEMALES WITH INVERSIONS INDICATED TO CROSSING OVER OBTAINED IN SUCH FEMALES WITHOUT INVERSIONS

INVERSIONS	AGE 2–6 DAYS y-w^{51a}	AGE 2–6 DAYS w^{51a}-*spl*	AGE 7–11 DAYS y-w^{51a}	AGE 7–11 DAYS w^{51a}-*spl*
Cy	2.2 ± 0.52	3.3 ± 0.97	1.8 ± 0.43	1.9 ± 0.44
Payne	2.8 ± 0.73	2.8 ± 0.82	2.0 ± 0.47	2.0 ± 0.47
Cy; Payne	6.0 ± 1.42	6.3 ± 1.69	4.6 ± 0.93	3.5 ± 0.73

inversions; together, $3^1/_2$ to over 6 times. It is of some interest that the higher ratios appear in each case in the first cultures; the relatively greater inversion

effects are exhibited by females of age 2–6 days as compared with females of age 7–11 days. Of more significance for the present study is the fact that within these limits of the increases produced by the inversions (1.8–6.3 times), and for six different types with respect to age and inversions, there is no preceptible tendency for the increases to be greater in a region which would seem a priori to have 20 times the physical length available for accommodating them.

For the sake of the record, brief mention may be made of a later experiment, which was quite incomplete compared with the above but in which the same stocks were used (for another purpose) and which gave a result reasonably compatible with the results reported above. Counts were made for first cultures only (mothers of age 2–6 days) and for two types only, that is, for control females without inversions and for females with combined *Curly* and *Payne* inversions. The control crossover values (N = 1,557) were 0.51 ± 0.18 for *y-w*^{51a} and 0.45 ± 0.17 for *w*^{51a}-*spl*. In the presence of *Curly* and *Payne* inversions (N = 4,535), crossing over increased to 5.0 ± 0.32 and to 3.5 ± 0.27, respectively. It might seem that these values show somewhat different rates of increase for the two subregions, since the ratio produced by dividing the *Curly-Payne* value by the control value is 9.8 for *y-w*^{51a} and is 7.8 for *w*^{51a}-*spl*. However the difference, 2, between these ratios has a standard error of 4.6. The discrepancy is no doubt introduced, first, through the very low numbers in the control and, second, through the low magnitude of the control values. In any case the ratios as they stand are definitely more nearly equal than they are in the proportion of 20 to 1.

A few apparent exceptions exist, in other work on this region, to a definite equality of capacity to increase. Thus Lewis[7] in his study of the pseudoalleles of the *white* locus found that *yellow-apricot* crossing over for attached-X females was, in the presence of the inversions of *Curly* and *Ultrabithorax*^{130}, 6 times standard value, whereas *white-split* reached only 1.7 times standard value; no control without inversions was included here. Also, unpublished data of the present author, from females heterozygous for *apricot* and either *buff* or a new reversion of *buff* to wild type, gave increases with various inversions which in some cases were somewhat greater in the *yellow-white* subregion than in *white-split*. However, both these sets of experiments were designed for other measurements and are for a number of reasons not completely acceptable for the present comparisons. This is particularly true because of the lack of proper controls; it is common experience that *yellow-split* does not, in the complete absence of inversions, uniformly exhibit standard crossing over but may differ from stock to stock, and in certain stocks shows definite differences between *yellow-white* and *white-split*. Thus it seems likely that these cases would fall into line under more controlled experimental conditions.

However, it should be emphasized that the significant point is not the precise equality of the increases of the two subregions, which is approached in the present experiment. It is rather that such marked increases are possible in the *white-split* subsection which is relatively physically so minute, and especially that the increases here are of the same order of magnitude as those in the adjacent *yellow-white* subsection, which though still small, is structurally of a definitely higher order of magnitude. The difference between the subregions is in this respect a qualitative one. Per unit of length, *yellow-white* may be considered more nearly saturated in its ability to develop an increase than is *white-split*.

The data from Table 1 can be presented somewhat differently in terms of what Bridges[8] called "coefficients of crossing over," which provide a simple numerical measure of the differences between cytological and genetic distances. A region is said to have a coefficient of crossing over greater than 1, or a lesser 1, if it has, per unit of length, a greater, or a lesser, tendency to cross over than does the average unit of length of all the chromosomes taken together. Bridges was concerned primarily with the right distal tip of chromosome II, in which crossing over had been believed to be generally high, and indeed he found that the simple distances here were relatively much greater for the linkage map than for his cytological map. He was further interested in the fact that the high coefficient of crossing over for this region was independent of a similarly computed low coefficient of mutation, as defined in terms of the number of detected workable mutations per unit of length.

Such coefficients of crossing over making use of the whole chromosomal complement are not applicable in the present experiment, because of the presence of the inversions, but one can make use of modified coefficients which compare the two regions with the average, not of all the chromosomes, but of the whole X only in the same inversion type. For this the previous data of Redfield (reported briefly by Morgan, Redfield, and Morgan[9]) are employed which deal with the effects of the *Curly* and *Payne* inversions for the entire X in y^2 *cv v f/ec car bb* females. The measure of cytological length of the X is preferably not the length in microns which was used by Bridges but the number of bands; this, as noted above, is presumably a less subjective unit, since, while salivary gland chromosomes show marked differential stretching, their banding is constant (i.e., in the absence of deficiencies, etc.). Actually, the relative values attained by the coefficients are not, for the *y-w* and *w-spl* regions, particularly affected by the method of measuring the cytological distances. It may be recalled that the crossover values shown in Table 1 for mothers without inversions are definitely substandard; hence the coefficients given in Table 3 are undoubtedly consistently lower than would be expected under "standard" conditions; however, there is no obvious reason to suppose that this affects the comparison between the coefficients of the two regions within a type. Table 3

TABLE 3

EFFECTS OF INVERSIONS ON COEFFICIENTS OF CROSSING OVER OBTAINED BY COMPARING VALUES FROM *y* w^{51a}*/spl* FEMALES WITH THOSE FOR THE ENTIRE X FROM y^2 *cv v f/ec car bb* FEMALES*

INVERSIONS	COEFFICIENT		INVERSIONS	COEFFICIENT	
	y-w^{51a}	w^{51a}-*spl*		*y*-w^{51a}	w^{51a}-*sp*
None	0.11	1.87	*Payne*	0.18	3.08
Cy	.18	3.59	*Cy; Payne*	.29	4.19

* See text.

shows that, for mothers without inversions, crossing over per band is only 0.1 times as great in the *y*-w^{51a} region as it is per band in the X taken as a whole; with the *Curly* or *Payne* inversions taken separately, it is about 0.2 times as great; and with both *Curly* and *Payne* inversions, it is about 0.3 times as great. However, for the w^{51a}-*spl* region the corresponding values are roughly 2.0, 3.0, and over 4.0; thus the coefficients are, for each type, approximately between 15 and 20 times as great in the second region as in the first. Under the circumstances of use of data from two entirely different experiments, this is of course close to the 20-to-1 expectation.

The tangential question may arise whether the observed increases are due purely to the presence of the heterologous inversions or whether they depend also upon genic differences—in the present case dominants introduced in the inversion chromosomes, since the chromosomes of all females are otherwise the same. Certain anomalous results of Carson[10] in *D. robusta* and of Levine and Levine[11] in *D. pseudoobscura* have been assumed to depend upon genic differences superimposed upon inversion effects. However, in these two species the experiments are more difficult to interpret than are those in *D. melanogaster*, where special precautions can be taken with respect to the comparability of the females. And although one cannot now deny the possibility of minor genic influences, still the agreement in the nature of the results for many different inversions and for various regions of all major chromosomes indicates that, in the latter species at least, the inversions themselves are the primary influences responsible for the increases (see review by Schultz and Redfield[3]).

It should be noted further that the present results cannot easily be explained as the simple corollary of a selective disappearance of noncrossovers and other low-rank crossover strands or of nondisjunctional types (although such is undoubtedly involved in the changed weight of classes in some recombination data; see the work of Sturtevant and Beadle[12] and the recent paper of Cooper, Zimmering, and Krivshenko[13]). The increases produced by heterologous inversions seem rather to be dependent upon a real facilitation of exchange between homologues, for multiples of an extremely high rank and doubles within a very short region are found, and these particular classes do not appear under other circumstances. It is not just a matter of simple shift in the proportions of already existing classes. Thus in work not included in the present paper three tested doubles from three mothers have appeared in the *y-w-spl* region in the presence of *Curly* and *Payne*, or *Payne* and the left inversion only of *Curly;* and such doubles have not occurred in the absence of inversions. Also, there has been one tested quintuple for the second chromosome in the presence of *ClB* and *Payne* inversions (crosses mentioned by Morgan, Bridges, and Schultz[14]). Steinberg[15] reported one quintuple for the X with the use of *Curly* and *Payne*, and the tables of Steinberg and Fraser[4] show no less than six quintuples in III in the presence of various X-chromosome inversions. These seem to be the only reported quintuples for *D. melanogaster*.

The suggestion sometimes made of an explanation in terms of oögonial or other earlier crossing over may be mentioned, although there is no positive indication of such phenomena in these data with heterologous inversions. The increases held by Whittinghill[16] to be the result of this type of aberrant recombination are, so far as the evidence goes, restricted to the centromere region. If oögonial crossing over occurs at all under the circumstances of the present experiment, distal sections such as the *yellow-split* region used here are not expected to show increased recombination values under its influence.

Of considerable interest in connection with the increases produced by inversions is the cytological work of Schultz and Hungerford[6] (abstract) carried out simultaneously with, and on the same material as, the present crossing-over study. Their paper (presented at the 1952 meeting of the Genetics Society of America and kindly made available to me by the authors) compares the tendency to synapse of the homologous X chromosomes in the $y\ w^{51a}/spl$ females of the four inversion types.

Direct observations were made on the frequency, length, and position of asynaptic regions in the X chromosomes of salivary gland cells. Statistically significant differences were found, in the pattern of somatic synapsis, between females containing both *Curly* and *Payne* inversions and females without inversions; the former gave in their X chromosomes a greater proportion of short intercalary failures of synapsis (For X chromosomes showing failures of pairing, 40 per cent of the failures were intercalary in females with *Curly* and *Payne*, as opposed to 17 per cent in females without inversions.) With the number of observations at hand it was not possible to establish a significant difference in this tendency for *Curly* and *Payne* taken separately. However, a further interchromosomal effect was definitely shown, in the presence of an extra set of autosomes, by the changed pairing of the two X chromosomes of intersexes (63 per cent intercalary versus the 17 per cent of the standard diploid). The cytological demonstration of the interchromosomal effects of the combined *Curly* and *Payne* inversions on the pattern of pairing of the X chromosomes has obvious possible bearing on increases in crossing over which are produced by interchromosomal effects on the X chromosomes by this same combination of inversions.

The specific mechanism responsible for the general pattern of interchromosomal effects cannot at this point be identified. It seems fairly clear that there is some relationship to such matters as the distribution of heterochromatin along the chromosome. The present results tell us a little about how the mechanism operates. They demonstrate in a controlled experiment, for a region of marked general increase, a constant difference in the capacity to react of very small adjacent subregions. One subregion which is 20 times the observed length of a second gives crossover increases which are equal to those of the second, and the increases produced remain equal for these two subregions for all the combinations of inversion and age types used. This suggests that the mechanism responsible for the increases in crossing over is distinct from the mechanism responsible for the local differences between linkage and cytological maps.

Summary.—Crossing over in the extreme left end of the X chromosomes in *y* w^{51a}/*spl* females was measured for four inversion types: those without inversions, those heterozygous for the *Curly* inversions of II, those heterozygous for the *Payne* inversions of III, and those heterozygous for both *Curly* and *Payne* inversions. The inversion chromosomes taken separately increased crossing over 2 to 3 times the value without inversions; together, $3^1/_2$ to over 6 times; younger females giving the higher ratios. For each inversion type, crossover values for *y*-w^{51a} and for w^{51a}-*spl* were increased equally within the limits of error; this was true both of first cultures (age of mothers 2–6 days) and of subcultures (age of mothers 7–11 days). Since the cytological lengths of these two subregions are known to be as 20 to 1, there was a marked difference in the response of the subregions to the heterologous inversions with respect to the increase in recombination per unit of cytological length. Coefficients of crossing over for the subregions remained approximately in the ratio of 1 to 20. Thus the short distal *yellow-split* region, a region of marked general increase in the presence of the inversions, can still be separated into subregions acting in a constant relationship to one another as regards their capacity to increase per unit of length. The suggestion is made that the mechanism producing the increases is different from that which results in the discrepancies between linkage and cytological maps.

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ABSTRACT HOMOTOPY. I

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1. *Introduction.*—Most theorems of homotopy theory, in particular those concerning homotopy and singular homology groups,[1] may be divided into two parts: (*a*) a theorem on abstract complexes and maps; (*b*) a "translation" of this abstract theorem into topological language by means of a singular functor (simplicial or cubical).

Such an abstract theorem, however, concerns only complexes which are the singular complex of a topological space. In this note it will be indicated how a homotopy theory may be developed for all abstract cubical complexes which satisfy only a certain *extension axiom;* homotopy groups will be introduced for all such complexes.

2. *Cubical Complexes.*[2]—The symbols ϵ and ω will always denote 0 or 1 even if indices are attached to them. A *cubical complex* K is a possibly void collection of elements σ (*cubes*) to each of which is attached a dimension $n \geq 0$ such that for each n-cube σ and integer i with $1 \leq i \leq n$ there are defined in K two $(n-1)$-cubes $\sigma 0^i$ and $\sigma 1^i$ (*faces*) and for each n-cube σ and integer j with $1 \leq j \leq n+1$ there is defined an $(n+1)$-cube $\sigma\eta^j$ of K (*degenerate*), where the operators 0^i, 1^i and η^j satisfy the following identities (we recall that $\epsilon = 0, 1$ and $\omega = 0, 1$):

$$\begin{aligned} \epsilon^i\omega^{j-1} &= \omega^j\epsilon^i, && i < j, \\ \eta^{j-1}\eta^i &= \eta^i\eta^j, && i < j, \\ \eta^j\epsilon^i &= \epsilon^i\eta^{j-1}, && i < j, \\ &= \text{identity}, && i = j, \\ &= \epsilon^{i-1}\eta^j, && i > j, \end{aligned} \tag{1}$$

A *subcomplex* M of K is a subcollection of K closed under the operators ϵ^i and η^j. A *cubical map* $f: K \to L$ is a dimension-preserving function which commutes with the operators ϵ^i and η^j. Let $\mathfrak{C}$ denote the resulting category.

A *tensor product* $K \otimes L$ can be defined which has a $(p+q)$-cube $\sigma \otimes \tau$ for every p-cube σ of K and q-cube τ of L, identifying the cubes $\sigma\eta^{p+1} \otimes \tau$ and $\sigma \otimes \tau\eta^1$. The operators ϵ^i and η^j are defined by

$$\begin{aligned} (\sigma \otimes \tau)\epsilon^i &= \sigma\epsilon^i \otimes \tau, & (\sigma \otimes \tau)\eta^j &= \sigma\eta^j \otimes \tau, & i, j &\leq p, \\ (\sigma \otimes \tau)\epsilon^i &= \sigma \otimes \tau\epsilon^{i-p}, & (\sigma \otimes \tau)\eta^j &= \sigma \otimes \tau\eta^{j-p}, & i, j &> p. \end{aligned}$$

Let I denote the cubical complex with a 1-cube ζ and two 0-cubes $\zeta_\epsilon = \zeta\epsilon^1$ as the only nondegenerate cubes; then two cubical maps $f_\epsilon: K \to L$ are called *homotopic* if there exists a cubical map $f_I: I \otimes K \to L$ (*homotopy*) such that $f_I(\zeta_\epsilon \otimes \sigma) = f_\epsilon\sigma$ for every cube σ of K (notation $f_I: f_0 \simeq f_1$ or $f_0 \simeq f_1$). This homotopy relation is reflexive but is not an equivalence relation.

A cubical complex K is called *connected* if for every two 0-cubes σ_ϵ of K there exists a 1-cube σ of K such that $\sigma\epsilon^1 = \sigma_\epsilon$.

Let $\partial\mathfrak{S}$ be the category of chain complexes. Then, as usual,[3] a functor F^N: $\mathfrak{C} \to \partial\mathfrak{S}$ can be defined as the quotient functor $F^N = F/F^D$, where F is obtained

by regarding all cubes of a cubical complex as generators of a chain complex and F^D by considering the subcomplex generated by the degenerate cubes. The functor F^N preserves tensor products. As all (co-)homological notions have originally been defined on the category $\partial\mathcal{G}$, it follows that by composition with the functor F^N these notions also supply to the category $\mathcal{C}$, and, as F^N preserves homotopies, the resulting (co-)homology theories satisfy the homotopy axiom.

3. *Notational Conventions.*—Consider the symbols

$$\begin{bmatrix} \sigma_0{}^1, \ldots, \sigma_0{}^n \\ \sigma_1{}^1, \ldots, \sigma_1{}^n \end{bmatrix} \quad (2), \qquad \begin{bmatrix} \tau_0{}^1, \ldots, \tau_0{}^p \\ \tau_1{}^1, \ldots, \tau_1{}^p \end{bmatrix} \quad (2'),$$

$$\left| \begin{matrix} \sigma_0{}^1, \ldots, \sigma_0{}^n \\ \sigma_1{}^1, \ldots, \sigma_1{}^n \end{matrix} \right| \quad (3), \qquad \left| \begin{matrix} \tau_0{}^1, \ldots, \tau_0{}^p \\ \tau_1{}^1, \ldots, \tau_1{}^p \end{matrix} \right| \quad (3'),$$

$$\begin{bmatrix} \sigma_0{}^1, \ldots, \sigma_0{}^l, \ldots, \sigma_0{}^n \\ \sigma_1{}^1, \ldots, x_1{}^l, \ldots, \sigma_1{}^n \end{bmatrix} \quad (4), \qquad \begin{bmatrix} \tau_0{}^1, \ldots, \tau_0{}^l, \ldots, \tau_0{}^p \\ \tau_1{}^1, \ldots, x_1{}^l, \ldots, \tau_1{}^p \end{bmatrix} \quad (4'),$$

where $\sigma_\epsilon{}^i$ and $\tau_\epsilon{}^i$ are $(n-1)$-cubes of a cubical complex K, and p is an integer with $1 \leq p \leq n$.

Symbol (2) will denote the existence of an n-cube σ of K such that $\sigma\epsilon^i = \sigma_\epsilon{}^i$ for all ϵ^i. Then σ is called a *solvent* of (2), and (2) is called the *boundary* of σ. Symbol (3) will denote an arbitrary but fixed solvent of (2). Symbol (4) is called an *equation* in an $(n-1)$-cube $x_1{}^l$ of K and will mean that $\sigma_\epsilon{}^i\omega^{j-i} = \sigma_\omega{}^j\epsilon^i$ for $i < j$ and $\epsilon^i, \omega^j \neq 1^l$. It is called *solvable* if there exists an $(n-1)$-cube $\sigma_1{}^l$ of K (a *solution*) such that (2) holds or, equivalently, if there exists an n-cube σ of K (a *solvent* of (4)) such that $\sigma\epsilon^i = \sigma_\epsilon{}^i$ for $\epsilon^i \neq 1^l$, for then $\sigma 1^l$ is a solution. The definition of an equation in an $(n-1)$-cube $x_0{}^l$ of K is similar.

Symbol (2′) will mean that (2) holds, where $\sigma_\epsilon{}^i = \tau_\epsilon{}^i$ for $i \leq p$ and $\sigma_\epsilon{}^i = \tau_0{}^1\eta^1\epsilon^i = \tau_1{}^1\eta^1\epsilon^i$ for $i > p$. An n-cube σ of K is called a solvent of (2′) if it is a solvent of (2). We call (2′) an (*abbreviated*) *boundary* of σ. An arbitrary but fixed solvent of (2′) will also be denoted by (3′). If $p = 1$, then we often write $[\tau_0{}^1, \tau_1{}^1]$ and $|\tau_0{}^1, \tau_1{}^1|$ instead of (2′) and (3′). Likewise, the symbol (4′), an (*abbreviated*) *equation*, will mean that (4) holds, where $\sigma_\epsilon{}^i = \tau_\epsilon{}^i$ for $i \leq p$, $\epsilon^i \neq 1^l$ and $\sigma_\epsilon{}^i = \tau_0{}^1\eta^1\epsilon^i$ for $i > p$. It is called *solvable* if equation (4) is so or, equivalently, if there exists an $(n-1)$-cube $\tau_1{}^l$ of K (*solution*) such that (2′) holds. The usefulness of these abbreviations may be seen from the following theorem.

THEOREM 1. *Let $\tau_\epsilon{}^i$ $(i = 1, \ldots, p$ and $\epsilon^i \neq 1^l)$ be $2p-1$ $(n-1)$-cubes of $K \in \mathcal{C}$ such that*

$$\tau_\epsilon{}^i\omega^{j-1} = \tau_\omega{}^j\epsilon^i, \qquad i < j,$$

$$\tau_\epsilon{}^i \text{ is a solvent of } \begin{bmatrix} \tau_\epsilon{}^i 0^1, \ldots, \tau_\epsilon{}^i 0^{p-1} \\ \tau_\epsilon{}^i 1^1, \ldots, \tau_\epsilon{}^i 1^{p-1} \end{bmatrix}.$$

Then (4′) holds. If, in addition, this abbreviated equation (4′) is solvable, then every solution has

$$\begin{bmatrix} \tau_0 1^{l-1}, \ldots, \tau_0{}^{l-1} 1^{l-1}, \tau_0{}^{l+1} 1^l, \ldots, \tau_0{}^p 1^l \\ \tau_1 1^{l-1}, \ldots, \tau_1{}^{l-1} 1^{l-1}, \tau_1{}^{l+1} 1^l, \ldots, \tau_1{}^p 1^l \end{bmatrix} \quad (5)$$

as an abbreviated boundary, i.e. (5) holds.

4. *The Extension Axiom.*—We shall need the following properties of a cubical complex K:

a) *Property* $E(n, \epsilon, i)$: Every equation in an $(n-1)$-cube $x_\epsilon{}^i$ of K is solvable.

b) *Property HE* (*homotopy extension*): Let $f_0: K \to L$ be a cubical map, let M be a subcomplex of K, and let g_I: $g_0 \simeq g_1$, where $g_0 = f_0 \mid M$. Then there exists a homotopy f_I: $f_0 \simeq f_1$ such that $f_I \mid I \otimes M = g_I$.

The strength of these properties may be indicated by the following theorems.

THEOREM 2. *A cubical complex K has the property HE if and only if it has the property $E(n, 1, 1)$ for all n.*

THEOREM 3. *If a cubical complex K has the property $E(n, 1, 1)$ for all n, then the homotopy relation $\simeq$ is an equivalence relation on the set of the cubical maps $L \to K$.*

THEOREM 4. *If a cubical complex K has the property $E(n, 0, n)$ for all n, then for every two q-cubes σ_ϵ of K there exists a $(q+1)$-cube σ such that $\sigma\epsilon^1 = \sigma_\epsilon$.*

We now define an *E-complex* as a cubical complex K which satisfies the following axiom:

Extension axiom: K has the properties $E(n, 1, 1)$ and $E(n, 0, n)$ for all n.

The full subcategory of $\mathfrak{C}$ generated by the E-complexes will be denoted by $\mathfrak{C}_E$. A map of $\mathfrak{C}_E$ is called an *E-map.*

THEOREM 5. *An E-complex K has the property $E(n, \epsilon, i)$ for all values of n, ϵ and i, i.e., every equation of K is solvable.*

5. *The Solutions of an Equation.*—Two n-cubes σ_ϵ of a cubical complex K are called *compatible*[4] if their faces coincide, i.e., if $\sigma_0\epsilon^i = \sigma_1\epsilon^i$ for all ϵ^i. They are called *compatible and homotopic*[4] if $[\sigma_0, \sigma_1]$, i.e., if there exists an $(n+1)$-cube σ of K such that $\sigma\epsilon^1 = \sigma_\epsilon$ and $\sigma\epsilon^i = \sigma_0\eta^1\epsilon^i$ for $i > 1$ (notation $\sigma_0 \sim \sigma_1$). This relation $\sim$ on the cubes of K is reflexive but need not be an equivalence relation. However, the following theorem holds.

THEOREM 6. *Let K be an E-complex. Then*

a) *The relation $\sim$ is an equivalence relation on the cubes of K,*

b) *The set of the solutions of an equation of K is exactly an equivalence class of cubes of K, and*

c) *This class depends only on the equivalence classes of the cubes $\sigma_\epsilon{}^i$ of the equation.*

The importance of this theorem lies in the fact that now symbols (2), (2′), (4), and (4′) may be considered as the definition of a kind of multimultiplication for the equivalence classes of n-cubes of an E-complex and that therefore these symbols still have a clear meaning if some of the entries are classes of n-cubes.

6. *The Homotopy Groups.*—Let ψ be an $(n\text{-}1)$-cube of an E-complex K. According to Theorem 6 the relation $\sim$ divides the solvents of $[\psi, \psi]$ into classes a, b, c, etc. Let 0 denote the class which contains $\psi\eta^1$. We now define the sum $a + b$ of two classes by the condition

$$\begin{bmatrix} a, & 0 \\ a+b, & b \end{bmatrix}. \tag{6}$$

It follows from Theorem 6 that the sum $a + b$ is uniquely determined for every a and b.

THEOREM 7. *The classes of the solvents of* $[\psi, \psi]$ *form a group* $\pi_n(K; \psi)$ *under the above definition of sum, the n-th homotopy group of K rel. the base-*$(n-1)$*-cube* ψ.

Proof: Let α, β, γ, $\alpha + \beta$, etc., be cubes of the classes a, b, c, $a + b$, etc. It follows from Theorem 5 that both equations

$$\begin{bmatrix} x_0{}^1, & \psi\eta^1 \\ \alpha, & \beta \end{bmatrix} \quad (7), \qquad \begin{bmatrix} \beta, & \psi\eta^1 \\ \alpha, & x_1{}^2 \end{bmatrix} \quad (7'),$$

are solvable, and therefore, in view of Theorem 6, the same applies to the equations $x + b = a$ and $b + y = a$. It now remains to prove that the associative law holds. As $\alpha\eta^1\epsilon^1 = \alpha$, $\alpha\eta^1\epsilon^2 = \alpha\epsilon^1\eta^1 = \psi\eta^1$, and $\alpha\eta^1\epsilon^i = \alpha\eta^1\epsilon^i$, it follows that, for $\alpha = \beta$, $\psi\eta^i$ is a solution of (7′) (i.e. 0 is a right zero). Now application of Theorem 1 to

$$\begin{bmatrix} \begin{vmatrix} & \alpha, & \psi\eta^1 \\ \alpha + \beta, & & \beta \end{vmatrix} & \begin{vmatrix} & \alpha, & \psi\eta^1 \\ & \alpha, & \psi\eta^1 \end{vmatrix} & \begin{vmatrix} & \psi\eta^1, & \psi\eta^1 \\ & \psi\eta^1, & \psi\eta^1 \end{vmatrix} \\ x_1{}^1 & \begin{vmatrix} & \alpha + \beta, & \psi\eta^1 \\ (\alpha + \beta) + \gamma, & & \gamma \end{vmatrix} & \begin{vmatrix} & \beta, & \psi\eta^1 \\ \beta + \gamma, & & \gamma \end{vmatrix} \end{bmatrix} \text{ yields } \begin{bmatrix} & \alpha, & \psi\eta^1 \\ (\alpha + \beta) + \gamma, & & \beta + \gamma \end{bmatrix},$$

i.e., $a + (b + c) = (a + b) + c$, *Q.E.D.*

It immediately follows from the definitions that an E-map $f\colon K \to L$ induces homomorphisms $f_*\colon \pi_n(K; \psi) \to \pi_n(L; f\psi)$ for all n. The isomorphisms of the homotopy groups of a topological space induced by a path generalize to isomorphisms $\gamma_*\colon \pi_n(K; \gamma 1^1) \to \pi_n(K; \gamma 0^1)$ induced by an n-cube γ of K by the condition

$$\begin{bmatrix} \gamma, & \gamma_* a \\ \gamma, & a \end{bmatrix}, \quad (8)$$

where $a \in \pi_n(K; \gamma 1^1)$. Application of Theorem 4 then yields

THEOREM 8. *Let* ψ, χ *be* $(n-1)$*-cubes of a connected E-complex K. Then there exists a not necessarily unique isomorphism* $\pi_n(K; \psi) \cong \pi_n(K; \chi)$.

The following can also be proved:

THEOREM 9. $\pi_n(K; \psi)$ *is Abelian for* $n > 1$.

For a set $\{\sigma\}$ of $2n + 2$ n-cubes $\sigma_\epsilon{}^i$ $(i = 1, \ldots, n + 1)$ of an E-complex K with $\sigma_\epsilon{}^i\omega^{j-1} = \sigma_\omega{}^j\epsilon^i$ for $i < j$, let $\alpha_0{}^1$ denote a solution of the equation in an n-cube $x_0{}^1$ of K involving the $\sigma_\epsilon{}^i$ except $\sigma_0{}^1$. We now define an element $c'\{\sigma\} \in \pi_n(K; \sigma_0{}^1 0^1)$ by the condition

$$\begin{bmatrix} \sigma_0{}^1, & c'\{\sigma\} \\ \alpha_0{}^1, & \sigma_0{}^1 1^1 \eta^1 \end{bmatrix}. \quad (9)$$

Using this and similar notions, the homotopy addition theorems[5] can be formulated and proved.

7. *Duality.*—Let $D\colon \mathfrak{C} \to \mathfrak{C}$ be the functor such that the cubical complex DK contains exactly one n-cube σ^* for every n-cube σ of K with operators $\sigma^* 0^i = (\sigma 1^{n+1-i})^*$, $\sigma^* 1^i = (\sigma 0^{n+1-i})^*$, and $\sigma^*\eta^j = (\sigma\eta^{n+2-j})^*$, and that for every cubical map $f\colon K \to L$ the map Df is determined by $(Df)\sigma^* = (f\sigma)^*$. Then, clearly, $DD = E$, the identity functor of $\mathfrak{C}$. It follows easily that a cubical complex K has the property $E(n, \epsilon, i)$ if and only if DK has the property $E(n, 1 - \epsilon, n + 1 - i)$. Consequently, the extension axiom is self-dual, i.e., K is an E-complex if and only if DK is so.

Dualizing the homotopy relation, we call two cubical maps f_ϵ: $K \to L$ *aft-homotopic* ($f_0 \overset{\sim}{\simeq} f_1$) if $Df_1 \simeq Df_0$. In general, the relations $\simeq$ and $\overset{\sim}{\simeq}$ do not coincide. However, we have

THEOREM 10. *Both homotopy relations coincide on* $\mathfrak{C}_E$.

[1] See, for example, Eilenberg and Mac Lane, *Ann. Math.*, **51**, 514–533, 1950, and Serre, *Ann. Math.*, **54**, 425–505, 1951.

[2] This is the cubical analogue of the complete semi-simplicial complexes of Eilenberg and Zilber, *Ann. Math.*, **51**, 499–513, 1950.

[3] Cf. Eilenberg and Mac Lane, *Am. J. Math.*, **75**, 189–199, 1953.

[4] Cf. Eilenberg and Zilber, *op. cit.*

[5] Sze-tsen Hu, *Ann. Math.*, **58**, 108–122, 1953.

ON THE IRREDUCIBLE REPRESENTATIONS OF THE SYMMETRIC GROUP

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We have pointed out[1] in a recent note in these PROCEEDINGS that all representations of S_n, the symmetric group on n symbols, are linear combinations with integral coefficients of appropriately symmetrized Kronecker powers of the irreducible representation $\Gamma(n-1, 1)$, of dimension $n-1$, of S_n and have furnished these linear combinations for the irreducible representations $\Gamma(n-p, \lambda_2, \ldots)$ of S_n in the special cases $p = 2, 3, 4$. In an accompanying note[2] we have given certain rules which facilitate (and in important instances furnish) the analysis of the Kronecker product of two irreducible representations of S_n into its irreducible components. The problem of determining this analysis is closely related to that of analyzing the various appropriately symmetrized powers of $\Gamma(n-1, 1)$, and the object of the present note is to indicate this connection and to extend the rules furnished in the note[2] just referred to. We also furnish the analysis of the various symmetrized powers $\Gamma(n-1, 1) \otimes \{\mu\}$, (μ) a partition of p, of $\Gamma(n-1, 1)$ in the cases $p = 5$ and $p = 6$.

With each partition $(\lambda) = (\lambda_1, \ldots, \lambda_K)$, $\lambda_1 \geqslant \lambda_2 \geqslant \ldots \geqslant \lambda_k$, of n there is associated an irreducible representation $\Gamma(\lambda)$ of S_n, and, in the Kronecker product $\Gamma(\lambda) \times \Gamma(\lambda')$ of two such irreducible representations, the passage from the partition (λ) to the associated partition (λ^*) of n acts like a change of sign of a factor in the product of two real or complex numbers (the product of $\Gamma(\lambda')$ by $\Gamma(\lambda^*)$ being the associate of the product of $\Gamma(\lambda')$ by $\Gamma(\lambda)$). On writing $\lambda_1 = n-p$, $p = 0, 1, \ldots, n-1$, (λ) appears as $(n-p, (\mu))$, where $(\mu) = (\lambda_2, \ldots, \lambda_k)$ is a partition of p, such that $n - p \geqslant \lambda_2$, and so we may regard the various irreducible representations of S_n as arranged in shells of varying depths, the number of representations in the shell of depth p being the number of those partitions of p whose first element $\leqslant n - p$. We may select from any pair of associated representations of S_n either of the two representations (the other being rejected as unessential), and we agree to select the representation of lesser depth. The effect of this selection is to reduce

the number of representations in some of the shells, the shells of sufficiently great depth being completely eliminated. For every n the superficial shell, that of depth 0, consists of the identity representation $\Gamma(n)$, and, since this acts as a unit in the product of representations, we may ignore it. The first effective shell, that of depth 1, consists of the representation $\Gamma(n-1, 1)$, of dimension $n-1$, which we shall denote, for brevity, simply by Γ. The second effective shell consists, if $n > 4$, of the representations $\Gamma(n-2, 2)$ and $\Gamma(n-2, 1^2)$, while if $n = 4$, it consists of the single representation $\Gamma(2^2)$, the representation $\Gamma(21^2)$ being rejected in favor of its associate $\Gamma(31) = \Gamma$. When $n = 3$, the second effective shell is empty, since $\Gamma(1^3)$, the only representation that could occur in it, is rejected in favor of its associate $\Gamma(3)$. We find it convenient to denote $\Gamma(n-p, (\mu))$ simply by (μ), so that $\Gamma(\lambda') \times \Gamma(\lambda) = \Gamma(n-p', (\mu')) \times \Gamma(n-p, (\mu))$ appears simply as $(\mu') \times (\mu)$. Since multiplication of representations is commutative, we may suppose that $p \geqslant p'$, i.e., that the depth of (μ) is not less than that of (μ'), and we do this. Then the various irreducible representations of S_n which appear in $(\mu') \times (\mu)$ all lie in the layer of shells whose minimum depth is $p - p'$ and whose maximum depth is $p + p'$. For example, the Kronecker product of any irreducible representation, of depth p, of S_n by Γ contains only representations of depths $p-1$, p, and $p+1$; in particular, the Kronecker square of Γ contains only representations of depths 0, 1, and 2. Actually, $\Gamma \times \Gamma = \Gamma(n-2, 2) + \Gamma(n-2, 1^2) + \Gamma(n-1, 1) + \Gamma(n)$, a result which appears in our abbreviated notation as $(1) \times (1) = (2) + (1^2) + (1) + (0)$. For $n = 2$, $(2) = \Gamma(02)$ cancels $(1) = \Gamma(1^2)$, while $(1^2) = \Gamma(01^2)$ vanishes, so that this reduces to $\Gamma(1^2) \times \Gamma(1^2) = \Gamma(2)$; for $n = 3$ it reduces to $\Gamma(21) \times \Gamma(21) = \Gamma(1^3) + \Gamma(21) + \Gamma(3)$, the term $(2) = \Gamma(12)$ vanishing, while, for $n > 3$, all four terms appear in the analysis. The determination of the irreducible components of $(\mu') \times (\mu)$ which appear in the shells at the fringes of the layer, i.e., in the shells of depths $p + p'$ and $p - p'$, is quite simple. In order to determine the components which appear in the shell of greatest depth, namely, $p + p'$, we proceed as follows: we form, by the well-known rule, the product of the irreducible representations $\{\mu\}$ and $\{\mu'\}$, of degrees p and p', respectively, of the n-dimensional linear group, this product being a linear combination $\sum_{(\nu)} c^{+}_{(\mu')(\mu)(\nu)} \{\nu\}$ of irreducible representations of degree $p + p'$ of the linear group. Then the components of $(\mu') \times (\mu)$ which lie in the deepest shell, i.e., the shell of depth $p + p'$, are furnished by the expression $\sum_{(\nu)} c^{+}_{(\mu')(\mu)(\nu)} (\nu)$. For example, since $\{2\}\{2\} = \{4\} + \{31\} + \{2^2\}$, the components of $(2) \times (2)$, i.e., $\Gamma(n-2, 2) \times \Gamma(n-2, 2)$, which lie in the shell of depth 4 are $(4) + (31) + (2^2)$, i.e., $\Gamma(n-4, 4) + \Gamma(n-4, 31) + \Gamma(n-4, 2^2)$. In order to determine the components of $(\mu') \times (\mu)$ which appear in the shell of depth $p - p'$, we resort to the less well-known process of *division* of irreducible representations of the linear group. If (μ) has j nonzero parts, the character of $\{\mu\}$ may be written, in the well-known way, as the determinant of a j-dimensional matrix whose diagonal elements are the complete symmetric functions $p_{\mu_1}, \ldots, p_{\mu_j}$ of the characteristic numbers of a typical element of the linear group; we denote by $\{\mu/\mu'\}$ the representation of the linear group whose character is the determinant of the j-dimensional matrix obtained by reducing the subscripts of the columns of the matrix whose determinant is the character of $\{\mu\}$

by $\mu_1', \ldots, \mu_{j'}'$, it being understood that $\{\mu/\mu'\}$ vanishes if $j' > j$ and that, if $j' < j$, $\mu_r' = 0$ if $r > j'$. Then $\{\mu/\mu'\}$ is a linear combination $\sum_{(\nu)} c^{-}{}_{(\mu')(\mu)(\nu)}\{\nu\}$ of irreducible representations of degree $p - p'$ of the linear group, and the components of $(\mu') \times (\mu)$ which lie in the least-deep shell, i.e., the shell of depth $p - p'$, are furnished by the expression $\sum_{(\nu)} c^{-}{}_{(\mu')(\mu)(\nu)}(\nu)$. It is easy to see that, if $p' = p$, $\{\mu/\mu'\}$ vanishes unless (μ') coincides with (μ), in which case $\{\mu/\mu'\}$ is $\{0\}$, the identity representation of the linear group, and that $\{\mu/1\} = \{\mu_1 - 1, \mu_2, \ldots, \mu_j\} + \ldots + \{\mu_1, \ldots, \mu_{j-1}, \mu_j - 1\}$. For example, $\{21/1\} = \{1^2\} + \{2\}$, so that the part of $(1) \times (21)$ which lies in the shell of depth 2 is $(2) + (1^2)$, the part which lies in the shell of depth 4 being $(31) + (2^2) + (21^2)$.

In order to determine the parts of $(\mu') \times (\mu)$ which lie in the shells immediately adjacent to the fringes of the layer, i.e., in the shells of depths $p + p' - 1$ and $p - p' + 1$, we use the following rules:

1. The part of $(\mu') \times (\mu)$ which lies in the shell of depth $p + p' - 1$ is furnished by $\{\mu'/1\}\{\mu/1\}\{1\}$; by this we mean that if $\{\mu'/1\}\{\mu/1\}\{1\} = \sum_{(\nu)} c_{(\mu'/1)(\mu/1)(\nu)}\{\nu\}$, then the part of $(\mu') \times (\mu)$ which lies in the shell of depth $p + p' - 1$ is $\sum_{(\nu)} c_{(\mu'/1)(\mu/1)(\nu)}(\nu)$.
2. The part of $(\mu') \times (\mu)$ which lies in the shell of depth $p - p' + 1$ is furnished by $(\{\mu/1\} \div \{\mu'/1\})\{1\}$, where we understand by a symbol such as $(a\{\mu\} + b\{\nu\}) \div (c\{\mu'\} + d\{\nu'\})$, for example, the sum $ac\{\mu/\mu'\} + ad\{\mu/\nu'\} + bc\{\nu/\mu'\} + bd\{\nu/\nu'\}$.

When $p' = 1$, so that $(\mu') = (1) = \Gamma$, both of these two shells coincide in the shell of depth p, the part of the analysis of $(1) \times (\mu)$ which lies in the central shell of the layer (i.e., the shell of depth p) being furnished by $\{\mu/1\}\{1\}$. Thus the analysis of $(1) \times (\mu) = \Gamma(n - 1, 1) \times \Gamma(n - p, \mu_1, \ldots, \mu_j)$ is completely furnished by the expression

$$\{1\}\{\mu\} + \{\mu/1\}\{1\} + \{\mu/1\}.$$

For example, $(1) \times (31^2) = (41^2) + (321) + (31^3) + (41) + (32) + 2(31^2) + (2^21) + (21^3) + (31) + (21^2)$, or, equivalently,

$$\Gamma(n - 1, 1) \times \Gamma(n - 5, 31^2) = \Gamma(n - 6, 41^2) + \Gamma(n - 6, 321) + \Gamma(n - 6, 31^3) + \Gamma(n - 5, 41) + \Gamma(n - 5, 32) + 2\Gamma(n - 5, 31^2) + \Gamma(n - 5, 2^21) + \Gamma(n - 5, 21^3) + \Gamma(n - 4, 31) + \Gamma(n - 4, 21^2).$$

It is remarkable that this formula is valid for all values of n; when $n = 8$, it furnishes the analysis of $\Gamma(71) \times \Gamma(3^21^2)$; when $n = 10$, it furnishes the analysis of $\Gamma(91) \times \Gamma(531^2)$, and so on.

When $p' = 2$, the rules we have given so far furnish the parts of the analysis of $(\mu') \times (\mu)$ which lie in the four shells of depths $p + 2$, $p + 1$, $p - 1$, and $p - 2$, but they do not complete the analysis of $(\mu') \times (\mu)$, since the part which lies in the central shell of the layer, i.e., the shell of depth p, is missing. To furnish this, we add the following rules:

3. The part of $(\mu') \times (\mu)$ which lies in the shell of depth $p + p' - 2$ is furnished by $\{\mu'/1\}\{\mu/1\} + (\{\mu'/2\}\{\mu/2\} + \{\mu'/1^2\}\{\mu/1^2\})\{2\} + (\{\mu'/2\}\{\mu/1^2\} + \{\mu'/1^2\}\{\mu/2\})\{1^2\}$.
4. The part of $(\mu') \times (\mu)$ which lies in the shell of depth $p - p' + 2$ is furnished by $(\{\mu\} \div \{\mu'/1\})\{1\} + [(\{\mu/2\} \div \{\mu'/2\}) + (\{\mu/1^2\} \div \{\mu'/1^2\})]\{2\} + [(\{\mu/2\} \div \{\mu'/1^2\}) + (\{\mu/1^2\} \div \{\mu'/2\})]\{1^2\}$.

When $p' = 2$, so that $(\mu') = (2)$ or (1^2), both of these two shells coincide in the shell of depth p, and the part of the analysis of $(2) \times (\mu)$ which lies in the central shell of the layer (i.e., the shell of depth p) is furnished by $\{1\}\{\mu/1\} + \{\mu/2\}\{2\} + \{\mu/1^2\}\{1^2\}$, while the part of the analysis of $(1^2) \times (\mu)$ which lies in this central shell is furnished by $\{1\}\{\mu/1\} + \{\mu/1^2\}\{2\} + \{\mu/2\}\{1^2\}$. Thus the analysis of $(2) \times (\mu) = \Gamma(n-2, 2) \times \Gamma(n-p, \mu_1, \ldots, \mu_j)$ is completely furnished by the expression

$$\{2\}\{\mu\} + \{\mu/1\}\{1\}^2 + \{\mu/1\}\{1\} + \{\mu/2\}\{2\} + \{\mu/1^2\}\{1^2\} + (\{\mu/1\} \div \{1\})\{1\} + \{\mu/2\},$$

and the analysis of $(1^2) \times (\mu) = \Gamma(n-2, 1^2) \times \Gamma(n-p, \mu_1, \ldots, \mu_j)$ is completely furnished by the expression

$$\{1^2\}\{\mu\} + \{\mu/1\}\{1\}^2 + \{\mu/1\}\{1\} + \{\mu/1^2\}\{2\} + \{\mu/2\}\{1^2\} + (\{\mu/1\} \div \{1\})\{1\} + \{\mu/1^2\}.$$

When $p' = 3$, so that $(\mu') = (3)$ or (21) or (1^3), the rules we have given so far furnish the parts of the analysis of $(\mu') \times (\mu)$ which lie in the six shells of depths $p+3, p+2, p+1, p-1, p-2, p-3$, but they do not complete the analysis of $(\mu') \times (\mu)$, since the part which lies in the central shell of the layer, i.e., the shell of depth p, is missing. To furnish this, we add the following rules:

5. The part of $(\mu') \times (\mu)$ which lies in the shell of depth $p + p' - 3$ is furnished by $(\{\mu'/1\} \div \{1\})(\{\mu/1\} \div \{1\})\{1\} + [\{\mu'/3\}\{\mu/3\} + \{\mu'/21\}\{\mu/21\} + \{\mu'/1^3\}\{\mu/1^3\}]\{3\} + [\{\mu'/3\}\{\mu/21\} + \{\mu'/21\}\{\mu/3\} + \{\mu'/21\}\{\mu/21\} + \{\mu'/21\}\{\mu/1^3\} + \{\mu'/1^3\}\{\mu/21\}]\{21\} + [\{\mu'/3\}\{\mu/1^3\} + \{\mu'/21\}\{\mu/21\} + \{\mu'/1^3\}\{\mu/3\}]\{1^3\}$.
6. The part of $(\mu') \times (\mu)$ which lies in the shell of depth $p - p' + 3$ is furnished by $(\{\mu/1\} \div \mu'/\{1\}^2)\{1\}^2 + [(\{\mu/3\} \div \{\mu'/3\}) + (\{\mu/21\} \div \{\mu'/21\}) + (\{\mu/1^3\} \div \{\mu'/1^3\})]\{3\} + [(\{\mu/3\} \div \{\mu'/21\}) + (\{\mu/21\} \div \{\mu'/3\}) + (\{\mu/21\} \div \{\mu'/21\}) + (\{\mu/21\} \div \{\mu'/1^3\}) + (\{\mu/1^3\} \div \{\mu'/21\})]\{21\} + (\{\mu/3\} \div \{\mu'/1^3\}) + (\{\mu/21\} \div \{\mu'/21\}) + (\{\mu/1^3\} \div \{\mu'/3\})]\{1^3\}$.

When $p' = 3$, both of these two shells coincide in the central shell, of depth p, of the layer of seven shells, and the parts of the analyses of $(3) \times (\mu)$, $(21) \times (\mu)$, $(1^3) \times (\mu)$ which lie in this central shell are furnished, respectively, by the expressions

$$(\{\mu/1\} \div \{1\})\{1\}^2 + \{\mu/3\}\{3\} + \{\mu/21\}\{21\} + \{\mu/1^3\}\{1^3\},\ 2(\{\mu/1\} \div \{1\})\{1\}^2 + \{\mu/21\}\{3\} + (\{\mu/3\} + \{\mu/21\} + \{\mu/1^3\})\{21\} + \{\mu/21\}\{1^3\},$$
$$(\{\mu/1\} \div \{1\})\{1\}^2 + \{\mu/1^3\}\{3\} + \{\mu/21\}\{21\} + \{\mu/3\}\{1^3\}.$$

Thus the rules given above furnish completely the analyses of $\Gamma(n-3,3)\times\Gamma(n-p,(\mu))=(3)\times(\mu)$, $\Gamma(n-3,21)\times\Gamma(n-p,(\mu))=(21)\times(\mu)$, and $\Gamma(n-3,1^3)\times\Gamma(n-p,(\mu))=(1^3)\times(\mu)$, and they furnish, when $p'>3$, the terms of $\Gamma(n-p',(\mu'))\times\Gamma(n-p,(\mu))=(\mu')\times(\mu)$ which lie in the eight outer shells of the layer, of thickness $2p'+1$, whose central shell has depth p. For example,

$$\begin{aligned}(3)\times(3) &= (6)+(51)+(42)+(3^2)+(5)+2(41)+2(32)+(31^2)+(2^21)\\ &\quad+2(4)+3(31)+2(2^2)+(21^2)+2(3)+2(21)+(1^3)+2(2)\\ &\quad+(1^2)+(1)+(0),\\ (21)\times(1^3) &= (321)+(31^3)+(2^21^2)+(21^4)+(41)+2(32)+3(31^2)+3(2^21)\\ &\quad+3(21^3)+(1^5)+(4)+4(31)+3(2^2)+5(21^2)+2(1^4)+2(3)\\ &\quad+5(21)+2(1^3)+2(2)+2(1^2)+(1),\\ (1^3)\times(1^3) &= (2^3)+(2^21^2)+(21^4)+(1^6)+(32)+(31^2)+2(2^21)+2(21^3)\\ &\quad+(1^5)+(4)+2(31)+2(2^2)+2(21^2)+(1^4)+2(3)+2(21)\\ &\quad+(1^3)+2(2)+(1^2)+(1)+(0).\end{aligned}$$

When $p'\geqslant 4$, the analysis of $(\mu')\times(\mu)$ may best be obtained by a method which we illustrate by means of the example $(4)\times(4)$. Using the rules given above, we obtain

$$\begin{aligned}(4)\times(4) &= (8)+(71)+(62)+(53)+(4^2)+(7)+2(61)+2(52)+(51^2)\\ &\quad+2(43)+(421)+(3^21)+2(6)+3(51)+4(42)+(41^2)+2(3^2)\\ &\quad+2(321)+(2^3)+2(5)+4(41)+3(32)+2(31^2)+(2^21)+a(4)\\ &\quad+b(31)+c(2^2)+d(21^2)+e(1^4)+2(3)+2(21)+(1^3)+2(2)\\ &\quad+(1^2)+(1)+(0),\end{aligned}$$

where a, b, c, d, e, are undetermined non-negative integers. Setting $n=7$, so that $(4)=\Gamma(3,4)$ vanishes, we obtain

$$\begin{aligned}0 = &-\Gamma(7)+\Gamma(51^2)+\Gamma(421)+\Gamma(3^21)-\Gamma(61)-2\Gamma(51^2)-\Gamma(41^3)+2\Gamma(32^2)\\ &+\Gamma(2^31)-2\Gamma(52)-3\Gamma(421)-4\Gamma(32^2)-\Gamma(321^2)-2\Gamma(2^31)-2\Gamma(43)\\ &-4\Gamma(3^21)+\Gamma(2^31)+b\Gamma(3^21)+c\Gamma(32^2)+d\Gamma(321^2)+e\Gamma(31^4)+2\Gamma(43)\\ &+2\Gamma(421)+\Gamma(41^3)+2\Gamma(52)+\Gamma(51^2)+\Gamma(61)+\Gamma(7),\end{aligned}$$

so that $(b-3)\,\Gamma(3^21)+(c-2)\,\Gamma(32^2)+(d-1)\,\Gamma(321^2)+e\Gamma(31^4)=0$ and $b=3, c=2, d=1, e=0$. To determine a, we set $m=4$, so that $(4)=\Gamma(0,4)=-\Gamma(31)=-\Gamma$ and $(4)\times(4)=\Gamma\times\Gamma=(2)+(1^2)+(1)+(0)=\Gamma(2^2)+\Gamma(21^2)+\Gamma(31)+\Gamma(4)$. We obtain at once $a=3$, so that the part of the analysis of $(4)\times(4)$ which lies in the central shell, of depth 4, is $3(4)+3(31)+2(2^2)+(21^2)$.

The analysis of $\Gamma\otimes\{\mu\}$, where (μ) is a partition of any integer p, is readily obtained from the fact that $(\Gamma\otimes\{\mu'\})\times(\Gamma\otimes\{\mu\})=\Gamma\otimes\{\mu'\}\{\mu\}$. For example, since $\Gamma\otimes\{1^{n-1}\}=(1^{n-1})$ and $\{1\}\{1^{n-1}\}=\{21^{n-2}\}+\{1^n\}$, we have $(\Gamma\otimes\{21^{n-2}\})+(\Gamma\otimes\{1^n\})=(1)\times(1^{n-1})=(21^{n-2})+(1^n)+(21^{n-3})+(1^{n-1})+(1^{n-2})$, so that $\Gamma\otimes\{21^{n-2}\}=(21^{n-2})+(21^{n-3})+(1^{n-1})+(1^{n-2})$. In other words, $\Gamma\otimes\{21^{n-2}\}$ is furnished by $\{21^{n-2}\}+\{1^{n-2}\}(\{1\}+\{0\})$. Similarly, $\Gamma\otimes\{2^21^{n-4}\}$ is furnished by $\{2^21^{n-4}\}+\{21^{n-4}\}(\{1\}+\{0\})+\{1^{n-4}\}(\{2\}+\{1\}+\{0\})$; $\Gamma\otimes\{2^31^{n-6}\}$ is furnished by $\{2^31^{n-6}\}+\{2^21^{n-6}\}(\{1\}+\{0\})+\{21^{n-6}\}(\{2\}+\{1\}+\{0\})+\{1^{n-6}\}(\{3\}+\{2\}+\{1\}+\{0\})$; $\Gamma\otimes$

$\{2^41^{n-8}\}$ is furnished by $\{2^41^{n-8}\} + \{2^31^{n-8}\}(\{1\} + \{0\}) + \{2^21^{n-8}\}(\{2\} + \{1\} + \{0\}) + \{21^{n-8}\}(\{3\} + \{2\} + \{1\} + \{0\}) + \{1^{n-8}\}(\{4\} + \{3\} + \{2\} + \{1\} + \{0\})$; and so on. When the first element of $(\mu) \geqslant 3$, we have been unable to determine rules of comparable simplicity for writing down $\Gamma \otimes \{\mu\}$. However, the maximum depth of the terms in $\Gamma \otimes \{\mu\}$ is p, (μ) being a partition of p, and the one and only term of depth p in $\Gamma \otimes \{\mu\}$ is (μ), while the terms of depth $p - 1$ are furnished by $\{\mu/2\}\{1\}$. The analyses of $\Gamma \otimes \{\mu\}$ for the various partitions (μ) of 5 and 6 are as follows (the analyses for the partitions of 2, 3, and 4 having been given in a preceding note[1]):

$$\Gamma \otimes \{1^5\} = (1^5); \qquad \Gamma \otimes \{21^3\} = (21^3) + (21^2) + (1^4) + (1^3);$$
$$\Gamma \otimes \{2^21\} = (2^21) + (31) + (2^2) + (21^2) + (3) + 2(21) + (2) + (1^2) + (1);$$
$$\Gamma \otimes \{31^2\} = (31^2) + (31) + (2^2) + 2(21^2) + (1^4) + 3(21) + 3(1^3) + (2) + 2(1^2);$$
$$\Gamma \otimes \{32\} = (32) + (4) + 2(31) + (2^2) + (21^2) + 3(3) + 4(21) + (1^3) + 4(2) + 3(1^2) + 3(1) + (0);$$
$$\Gamma \otimes \{41\} = (41) + (4) + 2(31) + (2^2) + (21^2) + 3(3) + 5(21) + 2(1^3) + 5(2) + 5(1^2) + 4(1) + (0);$$
$$\Gamma \otimes \{5\} = (5) + (4) + (31) + 3(3) + 2(21) + 4(2) + 3(1^2) + 4(1) + 2(0).$$
$$\Gamma \otimes \{1^6\} = (1^6); \qquad \Gamma \otimes \{21^4\} = (21^4) + (21^3) + (1^5) + (1^4);$$
$$\Gamma \otimes \{2^21^2\} = (2^21^2) + (31^2) + (2^21) + (21^3) + (31) + 2(21^2) + (21) + (1^3) + (1^2);$$
$$\Gamma \otimes \{2^3\} = (2^3) + (32) + (2^21) + (4) + (31) + 2(2^2) + 2(3) + (21) + 2(2) + (1) + (0);$$
$$\Gamma \otimes \{31^3\} = (31^3) + (31^2) + (2^21) + 2(21^3) + (1^5) + (2^2) + 3(21^2) + 3(1^4) + (21) + 2(1^3);$$
$$\Gamma \otimes \{321\} = (321) + (41) + 2(32) + 2(31^2) + 2(2^21) + (21^3) + (4) + 5(31) + 4(2^2) + 5(21^2) + (1^4) + 3(3) + 8(21) + 3(1^3) + 4(2) + 4(1^2) + 2(1);$$
$$\Gamma \otimes \{3^2\} = (3^2) + (41) + (32) + (31^2) + (4) + 4(31) + 2(21^2) + 3(3) + 4(21) + 3(1^3) + 2(2) + 5(1^2) + 2(1);$$
$$\Gamma \otimes \{41^2\} = (41^2) + (41) + (32) + 2(31^2) + (2^21) + (21^2) + 4(31) + 2(2^2) + 6(21^2) + 2(1^4) + 2(3) + 7(21) + 6(1^3) + 2(2) + 5(1^2) + (1);$$
$$\Gamma \otimes \{42\} = (42) + (5) + 2(41) + 2(32) + (31^2) + (2^21) + 4(4) + 6(31) + 5(2^2) + 3(21^2) + (1^4) + 8(3) + 11(21) + (31^3) + 11(2) + 6(1^2) + 7(1) + 3(0),$$
$$\Gamma \otimes \{51\} = (51) + (5) + 2(41) + (32) + (31^2) + 3(4) + 6(31) + 2(2^2) + 3(21^2) + 6(3) + 11(21) + 4(1^3) + 9(2) + 10(1^2) + 7(1) + (0);$$
$$\Gamma \otimes \{6\} = (6) + (5) + (41) + 3(4) + 2(31) + (2^2) + 5(3) + 4(21) + (1^3) + 8(2) + 4(1^2) + 7(1) + 4(0).$$

As a check upon the accuracy of the calculations, we have the relations

$$(1) \times (1) = (\Gamma \otimes \{2\}) + (\Gamma \otimes \{1^2\});$$
$$(1) \times (1) \times (1) = (\Gamma \otimes \{3\}) + 2(\Gamma \otimes \{21\}) + (\Gamma \otimes \{1^3\});$$
$$(1) \times (1) \times (1) \times (1) = (\Gamma \otimes \{4\}) + 3(\Gamma \otimes \{31\}) + 2(\Gamma \otimes \{2^2\}) + 3(\Gamma \otimes \{21^2\}) + (\Gamma \otimes \{1^4\});$$
$$(1) \times (1) \times (1) \times (1) \times (1) = (\Gamma \otimes \{5\}) + 4(\Gamma \otimes \{41\}) + 5(\Gamma \otimes \{32\}) + 6(\Gamma \otimes \{31^2\}) + 5(\Gamma \otimes \{2^21\}) + 4(\Gamma \otimes \{21^3\}) + (\Gamma \otimes \{1^5\});$$

$$(1) \times (1) \times (1) \times (1) \times (1) = (\Gamma \otimes \{6\}) + 5(\Gamma \otimes \{51\}) + 9(\Gamma \otimes \{42\}) + 10(\Gamma \otimes \{41^2\}) + 5(\Gamma \otimes \{3^2\}) + 16(\Gamma \otimes \{321\}) + 10(\Gamma \otimes \{31^3\}) + 5(\Gamma \otimes \{2^3\}) + 9(\Gamma \otimes \{2^2 1^2\}) + 5(\Gamma \otimes \{21^4\}) + (\Gamma \otimes \{1^6\}),$$

where

$$(1) \times (1) = (1)^2 + (1) + (0); \qquad (1) \times (1) \times (1) = (1)^3 + 3(1)^2 + 4(1) + (0);$$
$$(1) \times (1) \times (1) \times (1) = (1)^4 + 6(1)^3 + 13(1)^2 + 11(1) + 4(0);$$
$$(1) \times (1) \times (1) \times (1) \times (1) = (1)^5 + 10(1)^4 + 35(1)^3 + 55(1)^2 + 41(1) + 11(0);$$
$$(1) \times (1) \times (1) \times (1) \times (1) \times (1) = (1)^6 + 15(1^5) + 80(1)^4 + 200(1^3) + 256(1)^2 + 162(1) + 41(0)$$

and

$$(1)^2 = (2) + (1^2); \qquad (1)^3 = (3) + 2(21) + (1^3);$$
$$(1)^4 = (4) + 3(31) + 2(2^2) + 3(21^2) + (1^4);$$
$$(1)^5 = (5) + 4(41) + 5(32) + 6(31^2) + 5(2^2 1) + 4(21^3) + (1^5);$$
$$(1)^6 = (6) + 5(51) + 9(42) + 10(41^2) + 5(3^2) + 16(321) + 10(31^3) + 5(2^3) + 9(2^2 1^2) + 5(21^4) + (1^6).$$

Furthermore, it becomes clear, on setting $p = 0$, that, in all cases save the trivial one $\Gamma \otimes \{1^p\}$, the sum of the coefficients of (0), (1^2), (1^4), . . . , in $\Gamma \otimes \{\mu\}$ is the same as the sum of the coefficients of (1), (1^3), (1^5), ... Similarly, we see, on setting $p = 1$, that the sum of the coefficients of (0), (21), (21^3), . . . , in $\Gamma \otimes \{\mu\}$ is the same as the sum of the coefficients of (2), (21^2), (21^4), ...

The fact that the one and only term of depth p in $\Gamma \otimes \{\mu\}$ is (μ), there being no term of greater depth in $\Gamma \otimes \{\mu\}$, is the basis for the rule that the terms of greatest depth, namely, $p + p'$, in $\Gamma(n - p', (\mu')) \times \Gamma(n - p, (\mu))$ are furnished by the expression $\{\mu'\}\{\mu\}$. The rule furnishing the terms of least depth, namely, $p - p'$, in $\Gamma(n - p', (\mu')) \times \Gamma(n - p, (\mu))$ is, then, a consequence of the fact that if $\{\mu/\mu'\}$ contains $\{\nu\}$ k times, then $\{\mu'\}\{\nu\}$ contains $\{\mu\}$ k times.

The characters of the representations $\Gamma \otimes \{\mu\}$ of S_n are easily written down. For example, the character of $\Gamma \times \{2\}$ is $\frac{1}{2}(s_1^2 + s_2)$, where $s_1 = \alpha_1 - 1 = x$, say, is the character of Γ and $s_2 = \alpha_1 + 2\alpha_2 - 1 = x + 2\alpha_2$ is the character of Γ over the class which contains the squares of the elements of the class $(\alpha) = (\alpha_1, \alpha_2, \ldots, \alpha_n)$. Similarly, the character of $\Gamma \otimes \{1^2\}$ is $\frac{1}{2}(s_1^2 - s_2)$; that of $\Gamma \otimes \{3\}$ is $\frac{1}{6}(s_1^3 + 3s_1 s_2 + 2s_3)$; that of $\Gamma \otimes \{21\}$ is $\frac{1}{3}(s_1^3 - s_3)$; and that of $\Gamma \otimes \{1^3\}$ is $\frac{1}{6}(s_1^3 - 3s_1 s_2 + 2s_3)$, where $s_3 = \alpha_1 + 3\alpha_3 - 1 = x + 3\alpha_3$ is the character of Γ over the class which contains the cubes of the elements of the class (α). The character of $\Gamma \otimes \{4\}$ is $\frac{1}{24}(s_1^4 + 6s_1^2 s_2 + 8s_1 s_3 + 3s_2^2 + 6s_4)$, where $s_4 = \alpha_1 + 2\alpha_2 + 4\alpha_4 - 1 = x + 2\alpha_2 + 4\alpha_4$, and so on. From the relation $\Gamma \otimes \{1^2\} = (1^2)$, we see that the character of $(1^2) = \Gamma(n - 2, 1^2)$ is $\frac{1}{2}(x^2 - x - 2\alpha_2) = \frac{1}{2}x(x - 1) - \alpha_2 = \frac{1}{2}(\alpha_1 - 1)(\alpha_1 - 2) - \alpha_2$, and, since $(2) = (\Gamma \otimes \{2\}) - (1) - (0)$, we see that the character of $(2) = \Gamma(n - 2, 2)$ is $\frac{1}{2}(x^2 + x + 2\alpha_2) - x - 1 = \frac{1}{2}(x + 1)$ and $(x - 2) + \alpha_2 = \frac{1}{2}\alpha_1(\alpha_1 - 3) + \alpha_2$. For the deeper representations (μ) of S_n, however, this method of determining the characters becomes tedious, and that given in a previous note[3] is preferable.

[1] F. D. Murnaghan, "On the Generation of the Irreducible Representations of the Symmetric Group," these PROCEEDINGS, **41**, 514–515, 1955.

[2] F. D. Murnaghan, "On the Analysis of the Kronecker Product of Irreducible Representations of S_n," *ibid.*, pp. 515–518.

[3] F. D. Murnaghan, "On the Characters of the Symmetric Group," *ibid.*, pp. 396–398.

UNIQUENESS OF THE FINE-STRUCTURE CONSTANT

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1. We sketch here a direct and rigorous approach to the dynamics of fields of elementary particles. We hope at a later date to deal with specific interactions. In the present note it is deduced that under realistically general circumstances the coupling constant in a fully covariant interaction theory must be uniquely determined.[1]

The main novelty in this work is in its formulation of dynamics exclusively in terms of group representations, without the use, in principle, of Lagrangians or partial differential equations. This clarifies the covariance of the theory and leads in particular to a simple, unique method of going from a theoretically given particle to a corresponding field.

2. As is well known, there is associated with a given Lorentz-invariant partial differential equation a representation of the Lorentz group.[2] As indicated in part below, it appears that actually all the operationally significant physics of the particle described by such an equation may be extracted from a corresponding group representation. Hence "particle" may be defined provisionally as a group representation. The problem of formulating the structure of a field of such particles is then one of "quantizing" a group representation, rather than a partial differential equation.

The procedure for this, given below, requires only that the representation be unitary and continuous. Consequently, it is trivial to replace the Lorentz group by an arbitrary one in quantizing. In particular, the space-time co-ordinates of a relativistic particle may be included along with the various momenta by taking as the basic group that spanned by the co-ordinates x, y, z, and t, together with the generators of the Lorentz group and the unit operator. The fact that the commutator of any two such operators is a multiple of a third implies that there exists actually a group in the large of which the group described is the infinitesimal form.[3]

3. There are two mathematical features that seem essential for any quantum statistics: creation and annihilation operators, and transference of one-particle displacements to field displacements. Specifically, we may define a *quantum statistics* (C, Γ) over a (complex) Hilbert space $\mathfrak{H}$ (the "one-particle" space) as consisting of the suitable assignments (1) to each unit vector x in $\mathfrak{H}$ an operator $C(x)$ (the operation of "creating a particle with wave function x") on a Hilbert space $\mathfrak{K}$ (the "field" state space) and (2) to each unitary operator on $\mathfrak{H}$ (one-particle displacement) a unitary operator $\Gamma(U)$ on $\mathfrak{K}$ (field displacement).

It is physically appropriate to require that Γ give a continuous representation on $\mathfrak{K}$ of the group of all unitary operators on $\mathfrak{H}$. The relation to the occupation-

number formalism may be seen through the use of the infinitesimal representation $d\Gamma$ associated with Γ, which is defined as taking any self-adjoint operator A on $\mathfrak{H}$ into the self-adjoint generator of the one-parameter group $[\Gamma(e^{itA}); -\infty < t < \infty]$. The "number of particles with wave functions in the manifold $\mathfrak{M}$" is then $d\Gamma(P)$, where P is the projection with range $\mathfrak{M}$. It can be shown that for any representation Γ the proper values of such operators are integral and that they are non-negative in accordance with the occupation-number interpretation if and only if Γ is the direct sum of canonical representations of the unitary group on the spaces of covariant tensors over $\mathfrak{H}$, of maximal symmetry types.[4]

The properties characterizing the creation operator $C(x)$ are, notably, that it takes a state of the field y in which there are no x-particles into a state in which there is one x-particle, and that it is related to Γ through the equation.

$$\Gamma(U)\ C(x)\ \Gamma(U)^{-1} = C(Ux).$$

The statistics may be characterized as "elementary" in case no nontrivial invariant exclusion principles may be introduced, which may be formulated mathematically as the requirement that $\mathfrak{K}$ be irreducible under the joint action of the $C(x)$ and the $\Gamma(U)$. Under certain further assumptions it may be shown that there exist only two elementary quantum statistics, having as state spaces the spaces $\mathfrak{K}^+(\mathfrak{H})$ [or $\mathfrak{K}^-(\mathfrak{H})$] of all symmetric [or skew-symmetric] covariant tensors over $\mathfrak{H}$, as representation Γ^+ [or Γ^-] the canonical ones, and as creation operators $C(x)$ operations whose action on tensors of a fixed rank is proportional to the symmetrization [or skew-symmetrization] of tensor multiplication by x. These correspond to boson and fermion fields, respectively.[5]

The "free field" of particles described by the unitary representation U on $\mathfrak{H}$ of the group G and having, for example, Bose-Einstein statistics may now be defined as having the "kinematics" (or, equivalently in the free-field case, "dynamics") given by the representation $a \to \Gamma^+(U(a))$ on the state space $\mathfrak{K}^+(\mathfrak{H})$, where a is arbitrary in G. The infinitesimal generators of G are carried by the associated infinitesimal representation into the (integrated) field momenta, energy, etc. The "field" itself in the usual sense, after averaging with respect to the wave function x, is represented by suitable linear combinations of the $C(x)$ and their adjoints (cf. Cook, *op. cit.*,[5] and the reference there to Bohr and Rosenfeld).

4. Suppose, now, that we have given two interacting particles described by the unitary representations U_1 and U_2 of the basic group G, on Hilbert spaces $\mathfrak{H}_1$ and $\mathfrak{H}_2$. The kinematics of the coupled field may be roughly described as the dynamics of the uncoupled joint field, which is, however, a physical fiction. Alternatively, it may be described as the function giving the change in the state space of the coupled fields induced by a given change of frame in the underlying one-particle geometry. To be explicit, we take for definiteness the case when the particles obey Bose-Einstein and Fermi-Dirac statistics, respectively; the "kinematics" is then defined as the representation Γ_0 of G, where $\Gamma_0(a) = \Gamma^+(U_1(a)) \times \Gamma^-(U_2(a))$ and "$\times$" denotes the direct product. The infinitesimal form of Γ_0 carries the infinitesimal generators of G into the momenta, energy, etc., of the so-called "noninteracting" combined field.

The dynamics of the coupled field is given by the function Γ_T on G which describes how a transformation in G affects the actual physical field ("T" for "total,"

i.e., including interaction). By virtue of what may be called "phenomenological" covariance, applicable to any group-invariant theory, field-theoretic or otherwise, Γ_T is a representation of G. There is, in addition, the further type of covariance that is specifically field-theoretic and has the effect of relating the dynamics to the kinematics or, in other terms, connects the actual interacting motion with the hypothetical "noninteracting" motion. This "dynamical" covariance requires roughly that the interaction components of the dynamical variables of the coupled fields should be expressions in the fields that are independent of the frame of reference in the underlying one-particle geometry. Neither type of covariance implies the other type.

The simplest connection between Γ_T and Γ_0 arises from the fact that the states of the interacting fields are described in terms of the free-field states. This may be formulated by taking the state space of the coupled fields, i.e., the Hilbert space on which the $\Gamma_T(a)$ act, to be $\mathfrak{K}^+(\mathfrak{H}_1) \times \mathfrak{K}^-(\mathfrak{H}_2))$. In itself, however, this is not a significant restriction on Γ_T, for (i) any representation unitarily equivalent to Γ_T gives the same dynamics; (ii) in realistic cases the representation space of Γ_T will be denumerably dimensional, as will the spaces $\mathfrak{H}_1$ and $\mathfrak{H}_2$, and consequently also the space $\mathfrak{K}^+(\mathfrak{H}_1) \times \mathfrak{K}^-(\mathfrak{H}_2)$; (iii) any two Hilbert spaces of the same dimension are related by a unitary transformation. The substance of dynamical covariance is rather that the $\Gamma_0(a)$ and the $\Gamma_T(a)$ act on the same space in a suitably related manner. It is useful to formulate this relation in a precise and explicit way.

To arrive at such a formulation, we recall that in a standard version of field theory the interaction momentum-energy vector is expressed as an infinite polynomial in the annihilation and creation operators of the two particles. This fact in itself has little meaning, for any operator is in a formal sense such a polynomial, as the annihilation and creation operators constitute an irreducible set. However, the transformation properties of the expressions are significant, specifically the fact that they are covariant under changes of the Lorentz frame. This means, for example, that if a polynomial such as $\Sigma_{ijk} r_{ijk} C^{(1)}(e_i) C^{(2)}(f_j) C^{(2)}(f_k)^*$ is a term in the expression for the interaction energy $I(P_t)$, where P_t is the self-adjoint generator of translation in time, the $[e_i]$ and $[f_j]$ form orthonormal bases for $\mathfrak{H}_1$ and $\mathfrak{H}_2$, respectively, and the superscripts on the creation operators designate the particle, then the expression $\Sigma_{ijk} r_{ijk} C^{(1)}(U_1(a)e_i) C^{(2)}(U_2(a)f_j) C^{(2)}(U_2(a)f_k)^*$ is a term in the interaction component of the dynamical variable $I(aP_t a^{-1})$ of the field corresponding to the generator $aP_t a^{-1}$ of the Lorentz group, for an arbitrary Lorentz transformation a. Now because of the transformation property of $C(x)$ noted above, the transformed term may also be expressed as $\Gamma_0(a) I(P_t) \Gamma_0(a)^{-1}$. Thus the Lorentz invariance of the expression for the interaction momentum-energy vector is equivalent to the equations $\Gamma_0(a) I(P) \Gamma_0(a)^{-1} = I(aPa^{-1})$, where $I(P)$ denotes the interaction component of the dynamical variable of the coupled field corresponding to the generator P of a translation in space-time, and a is an arbitrary Lorentz transformation.

In general, if X is any infinitesimal generator of the group G describing the one-particle geometry, the interaction component $I(X)$ of the corresponding operator for the coupled fields may be unambiguously defined as $d\Gamma_0(X) - d\Gamma_T(X)$, where $d\Gamma_0$ and $d\Gamma_T$ are the infinitesimal representations associated with Γ_0 and Γ_T, respectively, and the negative of the customary interaction term is used, for reasons

that appear shortly. The discussion in the preceding paragraph leads to the following:

> *Definition:* The total motion Γ_T is *dynamically covariant* in case, for any transformation a of the basic one-particle group G and any infinitesimal generator X of G, the interaction component $I(X)$ of the dynamical variable of the coupled fields corresponding to X transforms as follows:
>
> $$I(aXa^{-1}) = \Gamma_0(a)I(X)\Gamma_0(a)^{-1}.$$

It can be seen purely mathematically that dynamical covariance implies (and in the case of a connected group is implied by) the equations $I([Y, X]) = i[I(X), I(Y)]$, for any generators X and Y of G, where the bracket denotes the commutator. This means that I must give an infinitesimal representation of G.

5. Invariance under gauge transformations and charge conjugation are, broadly speaking, special cases of the types of covariance already treated. Suitable gauge invariance for coupled fields may be defined as the specialization of the dynamical covariance conditions to the case when the group element a inducing the inner automorphism is in the subgroup whose infinitesimal generators are the space-time co-ordinates x, y, z, and t. The covariant elimination of the longitudinal waves in the quantization of the electromagnetic field is automatic from the restriction of the state space involved in obtaining a unitary representation.

Invariance under charge conjugation is the special case of dynamical covariance in which the transforming element a is time reversal. To accommodate this element, our earlier definitions are extended by allowing the representation operators to be either unitary or the product of a unitary operator with a conjugation (i.e., a real-linear transformation J such that $J = J^{-1}$ and $J(ix) = -iJ(x)$ for arbitrary vectors x). In dealing with these more general representations, it is often convenient to formulate the covariance condition in global terms, as the equation

$$\Gamma_T(aba^{-1}) = \Gamma_0(a)\,\Gamma_T(b)\,\Gamma_0(a)^{-1}.$$

6. In the standard theory of the coupled electromagnetic and electron-positron fields[6] the energy-momentum tensor depends in an explicitly linear fashion on the coupling constant g but also depends in an implicit fashion on it, through the dependence of the fields themselves on g. However, the commutation and anticommutation relations have no explicit dependence on the coupling constant, so that the implicitly g-dependent field can be obtained from a fixed g-independent field through transformation by a g-dependent unitary operator. Denoting the operators of the fixed field by primes, the relations $I'([X, Y]) = [I'(X), I'(Y)]$ hold for all admissible values of g, as such relations are preserved under unitary transformation. The total dependence of $I'(X)$ on g is linear at least when X is a linear or angular momentum, or the energy, generator, by virtue of the usual determination of the basic dynamical variables from the energy-momentum tensor. Substituting an arbitrary linear momentum for X and angular momentum for Y, it follows that if there is more than one nonzero admissible value for g, then all the interaction linear momenta must vanish. From this it follows from the definition of dynamical covariance that the interaction energy must vanish, which absurdity establishes the uniqueness of g. The same reasoning applies to any fully (i.e., both

phenomenologically and dynamically) covariant theory of interaction between two particles, in which the explicit dependence of the basic field dynamical variables on the coupling constant is linear.

7. Besides providing a possible basis for treating other groups G than that of the standard relativistic theory, the present approach may aid in determining theoretically which pairs of elementary particles can admit nontrivial interactions. The existence of a nontrivial representation Γ_T satisfying the condition for dynamical covariance imposes a significant restriction on the representations U_1 and U_2 defining the two elementary particles in question. The same is true of interactions of any finite number of types of elementary particles. The technical problems that arise in the development of this application appear to require a comprehensive mathematical theory of tensor invariants of unitary representations of Lie groups.

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[1] The present work is for the most part a summary of a portion of a course given at the University of Chicago during summer, 1955, notes on which are in process of duplication.

[2] See, in particular, V. Bargmann and E. P. Wigner, these PROCEEDINGS, **34**, 211–223, 1948, where further references are given.

[3] Difficulties in the definition of satisfactory space-time co-ordinates for elementary relativistic particles are treated at length in the notes mentioned above (n. 1). The "elementarity" of the particle is reflected by the "factorial" character of the corresponding representation, i.e., by the feature that there is no nontrivial subspace invariant under both the representation operators and all the operators commuting with them. However, this definitive aspect of elementarity plays no part in the present note.

[4] For the infinite-dimensional case that is realistically relevant, this extension of an essentially well-known finite-dimensional result (see H. Weyl, *The Classical Groups* [Princeton, N. J.: Princeton University Press, 1939]) is proved by the author in a paper submitted to a mathematical journal. A major part of the determination of the admissible quantum statistics is in a second such paper.

[5] These fields have been treated in terms of tensors by J. M. Cook (*Trans. Am. Math. Soc.*, **74**, 222–245, 1953). Throughout this note the superscripts "+" and "−" refer to the type of statistics, while the adjoint of an operator is indicated by an asterisk.

[6] See, e.g., Julian Schwinger, *Phys. Rev.*, **74**, 1439–1461, 1948.

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